PHOTO-ACTIVATED DISINFECTION OF THE ROOT CANAL SYSTEM

Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment for the Degree of

MASTER OF DENTAL SURGERY

BRANCH IV

CONSERVATIVE DENTISTRY AND ENDODONTICS

MARCH 2010

CERTIFICATE

This is to certify that this dissertation titled **"PHOTO-ACTIVATED DISINFECTION OF THE ROOT CANAL SYSTEM"** is a bonafide record of work done by **DENNIS MOHAN** under our guidance during the study period between **2007-2010.**

This dissertation is submitted to **THE TAMIL NADU Dr. M.G.R. MEDICAL UNVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

Guided by:

Dr. Revathi Miglani, M.D.S, DNB.,
Associate Professor,
Department of Conservative
Dentistry and Endodontics,

Dr. R. Indira, M.D.S.,
Professor and H.O.D,
Department of Conservative
Dentistry and Endodontics,

Dr. S. Ramachandran, M.D.S.,
Professor and Principal,
Department of Conservative Dentistry and Endodontics
Ragas Dental College and Hospital, Chennai

ACKNOWLEDGEMENT

I would like to take this opportunity to sincerely thank my post graduate teacher and guide ***Dr. Revathi Miglani, M.D.S, DNB, Associate Professor,*** *Department of Conservative Dentistry & Endodontics, Ragas Dental College & Hospital, for her perseverance in motivating and supporting me throughout my study period.*

I extend my sincere thanks to my post graduate teacher ***Dr. R. Indira, M.D.S, Professor and HOD****, Department of Conservative Dentistry & Endodontics, Ragas Dental College & Hospital, for her continuous guidance, support, constant encouragement and motivation throughout my postgraduate curriculum.*

My sincere thanks to ***Dr. S. Ramachandran M.D.S, Professor and Principal,*** *Department of Conservative Dentistry & Endodontics, Ragas Dental College & Hospital, who has helped with his advice and immense support and motivation throughout my postgraduate curriculum.*

My sincere thanks to my post graduate teacher ***Dr. P.Shankar, M.D.S****,* ***Professor****, Department of Conservative Dentistry & Endodontics , Ragas dental college & Hospital, who has helped with*

his advise and immense support throughout my postgraduate curriculum

My sincere thanks to my post graduate teacher ***Dr. R. Anilkumar, M.D.S****,* ***Professor****,* ***Dr. C. S. Karumaran M.D.S, Professor,****Department of Conservative Dentistry & Endodontics , Ragas dental college & Hospital, for his continuous guidance and constant encouragement throughout my study period.*

*I would like to solemnly thank****, Dr. M. Rajasekaran M.D.S, Associate Professor, Dr. Veni Ashok M.D.S, Dr. A.D. Senthil Kumar M.D.S, Dr. Sathya Narayanan M.D.S, Dr. D. Duraivel M.D.S, Dr. Venkatesan M.D.S and Dr. Shankar Narayan M.D.S, Senior lecturers*** *for all the help during my study period.*

I am extremely indebted to ***Dr. M. R. Srinivasan, M.D.S, Professor,*** *Department of Conservative & Endodontics, Sri Venkateshwara Dental College, who helped me with his valuable advice and immense support wherever and whenever needed*

I am extremely thankful to ***Dr.Ravanan****, for his guidance in biostatistics.*

I am extremely thankful to ***Dr P.K Gupta****, Head of Dept, Dept of Biomedical Application and* ***Dr Mrinalini Sharma*** *Laser Biomedical Application RRCAT, Indore in giving me permission to*

use the lab facilities and who helped me in conducting the experiment.

I am profoundly thankful to ***Mrs. K. Mahalakshmi, Research scholar****, Department of Microbiology, Institute Of Basic Medical Sciences for guiding and encouraging me throughout the study.*

I remain ever grateful to all ***my batch mates, my post graduate colleagues and friends*** *for their eternal support.*

I would like to especially thank ***my father, my mother, my brother, my wife and my son*** *for their love, understanding, support and encouragement throughout these years without which, I would not have reached so far.*

I also wish to thank the management of ***Ragas Dental College and Hospital,*** *Chennai for their help and support.*

Above all, I am thankful to ***God****, who always guides me and to have given these wonderful people in my life.*

CONTENTS

INTRODUC TION

The main goal of endodontic treatment is the complete debridement of the root canal system to eliminate all bacteria, bacterial by-products, and tissue debris from root canal system. Treatment procedures to eliminate the infection include chemo mechanical preparation and sealing of the root canal system with an inert material to provide fluid impervious seal. The main cause of treatment failures is the presence of persistent microorganisms and their recontamination of canals.[17]

The most frequent genera of microorganisms isolated from root canal with necrotic pulp *were Prevotella, Fusobacterium, Clostridium Lactobacillus, Streptococcus, , Peptostreptococus* and *E-Faecalis.* These bacteria are predominantly anaerobic and gram positive. On the other hand microbial findings of failed endodontic treatment have reported a very limited assortment of microorganism with predominantly facultative anaerobic gram positive species, especially *E-Faecalis* and fungi such as *candida albicans.*[14]

It has been suggested that *E-Faecalis* virulence may be related to resistance to intracanal medicaments and an ability to survive in

the root canal as a single organism without the support of other bacteria.[37]

Complete chemo-mechanical preparation is considered an essential step in root canal disinfection. The primary objectives of chemo-mechanical preparation are removal of infected hard tissue, use of irrigants for disinfection, to create space for delivery of medicaments and subsequent obturation. Chemo-mechanical preparation includes debriding the infected dentinal walls of root canal system using files in a sequence and use of irrigants. Ideal irrigant or combination of irrigants kills bacteria, dissolves necrotic tissue, lubricates canal, removes smear layer and does not irritate healthy tissue. Most commonly used irrigants are 2.5% to 5.2%,sodium hypochlorite, 0.2% chlorhexidine, 15% to 17% EDTA, hydrogen peroxide.[49] Despite all these techniques, total elimination of the bacteria may be difficult to accomplish.

The local micro environment of root canals favours few bacterial species that can survive and proliferate when they are out of reach of hosts immune response. Rinsing solution used with conventional root canal treatment could eliminate those bacteria only partially. In addition bacteria such as *E-Faecalis* are able to form intra and

extra radicular biofilims, which makes it even harder to control them. Despite the improvement in instrumentation techniques and use of intracanal medicaments, failure of endodontic treatment is reported in the literature. [49] Considering this, disinfection of root canal, including the most distant areas of the tubular system is a major challenge in endodontic treatment and is of fundamental importance for the success of endodontic treatment.[32]

Contemporary treatment procedures include use of ultrasonics along with NaOCl and lasers. Ultrasonically activated files have the potential to prepare and debride root canals mechanically. Lasers also have been commercially available for use in dentistry since 1990 and the use of lasers in the field of endodontics is an innovative approach for meeting these requirements. In general dental laser provide access to unreachable parts of the tubular network, owing to the fact that they penetrate dental tissue better than rinsing solutions, consequently have ancillary antimicrobial effects to aid in the reduction of bacteria in the root canal. [32]

Low power lasers within visible region along with dyes or Photosensitiser (PS) have been used recently for root canal disinfection, which is termed as photo activated disinfection(PAD) .

PAD is a newer antimicrobial strategy that involves the combination of a non toxic photosensitizer(PS) or dyes and a non harmful visible light source to disinfect root canal. Low power laser in itself is not particularly lethal to bacteria, but is useful for photochemical activation of oxygen- releasing dyes. Singlet oxygen released from dyes causes membrane and DNA damage to micro-organisms. PAD technique can be undertaken with a range of visible red and near infrared lasers and a dye such as toluidine blue, methyline blue, chlorine p6 etc. [26]

The aim of the present study was to explore the efficacy of photo-activated disinfection in reducing the CFU's of *E-Faecalis.*

The objective of the study was to compare the efficacy of photo-activated disinfection with conventional endodontic treatment and also a combination of conventional endodontic treatment along with photo-activated disinfection.

REVIEW OF LITERATURE

LJ Walsh (1997)[45] reviewed that there has been increasing interest in tooth-related or hard tissue application of low level laser theraphy. This article provided an overview of applications of low level laser theraphy in the treatment of dentine hypersensitivity and pain arising from the periodontal ligament and described the phenomenon of lethal laser photosensitization and its application in the treatment of dental caries.

Nicholas and Soukos et al(1998)[40] explored a new approach for antimicrobial therapy with light activation of targeted poly –l-lysine –chlorine e6 conjugates .The goal was to test the hypothesis that these conjugates between pL and ce6 would efficiently target photo destruction against gram positive and gram negative oral species while sparing an oral epithelial cell lines. Results showed by the selective uptake by bacteria(20-100 folds) compared to that by mammalian

cells while free ce6 showed much less selectivity for bacteria (5-20 folds).

Roger M Clarkson et al (1998)[9] reviewed the chemical properties and production of commercial NaOCl. It was concluded that NaOCl are effective endodontic irrigants over a wide concentrations(1% to 5.25%). NaOCl is both an oxidizing and hydrolyzing agent,it is bacteriocidal and proteolytic in nature and has been used as an endodontic irrigant as early as 1920.

Mark Wainwright et al in (1998)[47] reviewed the use of photodynamic antimicrobial therapy.They concluded that major advances have been made in photodynamic antimicrobial chemotherapy in the past decade ,while the early stages of this research has been mainly concerned with the disinfection of whole blood and blood products, the development of spin –off regimens involve with the irradication of localized infection by pathogenic microorganism.

J.F. siqueira Jr et al in (1999)[37] reviewed the antimicrobial activity of calcium hydroxide. It was concluded that calcium hydroxide has a limited antimicrobial spectrum that does not affect all members of the endodontic microbiota. In addition ,physiochemical properties of this substance may limit its effectiveness in disinfecting the entire root canal system after a short term use.

David kessel and Luo(1999)22 examined factors relating to the initiation of rapid apoptopic response by photodynamic therapy.PDT is a FDA approved form of cancer therapy involving the some what selective photoactivation of neoplastic cells usually with porphrin or porphyrin like drugs. The results were mainly acquired using porphycene PcM but identical results were obtained with CPO and resulted in 90% reduction of cell viability from photodynamic effects.

CH Sibata et al (2000)[38] discussed the present status of clinical Photodynamic therapy with newer photosensitizer.Despite the promising results from earlier clinical trials, considerable additional work is needed to bring this new modality of treatment into clinical practice. Improvements in the lioght source delivery, light dosimetryand computation of models of treatment are necessary to standardize treatments.

Y Kimura et al (2000)[23] concluded that with development of thinner more flexible and durable laser fibres ,laser application in endodontics will increase.since laser devices are relatively costly acess to them is limited.Once our knowledge of optimal parameters for each treatment modiality is complete,laser scan be developed that will provide dentist with the ability to care for patients with improved techniques and equipment.

RM Love (2001)[27] *et al* did a study to identify the possible mechanism that could explain how *E-Faecalis* could survive

and grow within dentinal tubules and reinfect an obturated root canal.It was concluded that virulence factor of *E.Faecalis* in failed endodontically treated teeth may be related to the ability of *E.Faecalis* cells to maintain the capability to invade dentinal tubules and adhere to the collagen in presence of human serum.

Lana MA et al (2001)[25] microbioligically analysed 31 canals with pulp necrosis before and after manipulation .Obligate and facultative anaerobes, microaerophilic bacteria and yeast were recovered from 24, 14, 5 and 2 clinical specimen respectively .The most frequent genera were *prevotella, fusobacterium, lactobacillus, streptococcus, clostridium and peptostreptococcus* for bacteria *and candida and saccharomyces* for yeast.

JF Siqueira Jr (2001)[39] studied the etiology of failure of root canal treatment particularly in cases of well root canal .It was concluded that persistent intra radicular or secondary infections, in some cases extraradicular infection are the

major causes of failure of both poorly treated and well treated root canal.

I J Macdonald et al (2001)[29] concluded that photofrin PDT has been approved in use in U.S. against advanced stage oesophagal, small lung cancer. Photofrin PDT has been approved for use in 5 European countries, Canada and Japan. Photofrin PDT is also being investigated has an adjunct therapy for treatment of the surgical bed after recession of malignant mesothelioma, head and neck cancer and intra peritoneal cancer.

GJ Seal (2002)[33] compared the bacterial killing of streptococcus intermedius biofilms in root canal using lethal photosensitization with various combination of photosensitizer concentration and lethal dose or 3% sodium hypochlorite irrigation.

Alexandra Almyroudi et al (2002)[3] compared the suitability of four disinfectants as intracanalmedications: calcium hydroxide,

chlorhexidine gel,chlorhexidine in the form of a controlled-release delivery system (PerioChip), and the combination of chlorhexidine gel with calcium hydroxide. Saline was used as the control.Results showed that the combination of chlorhexidine gel with calcium hydroxide and chlorhexidine gel works slightly better than the perio chip but there was no significant difference among the medications.

LJ Walsh et al (2003)[46] summarized the current and emerging application for lasers in clinical practice. A major application of low power lasers is the detection of caries, using fluorescence elicited from hydroxyapatite of from bacterial by products. Laser fluorescence is an effective method for detecting and quantifying incipient occlusal and cervical lesions. Photoactivated dye techniques have been developed which use low power laser to elicit a photochemical reaction. Photoactivated disinfection can be used to disinfect root canals,periodontal pockets, cavity preparations and sites of peri-implantitis. Laser can also be used for bleaching of tooth.

Nikolaos S Soukos (2003)[41] investigated photodynamic effect of a conjugate between the photosensitizer ce6 and polylysin with 5 lysin residue on human dentin plaque bacteria as well as on biofilms of oral species after their exposure to photomechanical waves generated by the presence of a laser in presence of a conjugate.Results achieved killing of almost 99% after photodynamic therapy and so PW assisted photo-destruction of dental plaque may be a potentially powerful tool for treatment of chronic destructive periodontal disease.

Mrinalini sharma et al (2004)[34]studied the effect of ph on uptake and photodynamic action of chlorine p6 on colon and breast adenocarcinoma cells and it was found that no significant variation in uptake , photosensitivity and sites of photo damage was observed for MCF cells at different extracellular ph and lower photosensitivity of lysosomes as compared to mitochondria in these cells suggest chlorine p6 is taken up through diffusion rather than endocytosis.

Michael T Lee (2004)[26] reviewed an alternate approach to microbial killing in root canal system with laser light involves the use of low power lasers to drive a photochemical reaction that produces reactive oxygen species, by technique termed photoactivated disinfection . By using exogenous photosensitizer ,killing of all type of bacteria can be achieved. Invitro studies of PAD demonstrated its ability to kill photosensitize oral bacteria (such as *E.faecalis*)and more recently microbial killing in vivo in root canal system has been demonstrated while PAD can be undertaken as part of routine disinfection of root canal system, it has the potential use for eradicating persistent endodontic infections for which conventional methods has been unsuccessful.

Michael R Hamblin (2004)[21] suggested that photodynamic therapy employs a non toxic dye termed a photosensitizer and low intensity visible light which in the presence of oxygen , combine to produce cytotoxic species .PDT has the advantage of dual sensitivity is that, PS can be targeted to its destination cell or tissue and in addition illumination

can be spatially directed to the lesion . PDT has been previously used to kill pathogenic microorganism invitro. Possible future clinical application include infection in wounds and burns rapidly spreading soft tissue abcess and infections , infections in body cavities such as mouth ,ear , nasal sinus, bladder and stomach and surface infection of cornea and skin.

Ronn R Allison et al (2004)[2] reviewed that photosensitizers in PDT allow for the transfer and translation of light energy into a type 2 chemical reaction.In clinical practice photosensitizer may arise from three families namely porphyrins ,chlorophylls and dyes. All clinically successful photosensitizer have the ability to a greater or lesser degree,to target specific tissue or there vasculature to achieve ablation . Each photosensitizer needs to reliably activate at a high enough light wavelength useful for therapy . This review looked at the potential benefits and consequence of each major photosensitizer that has been tried in clinical setting.

Mrinalani Sharma et al (2005)[35] investigated the effect of varying extracellular ph on mode of cell death induced by photodynamic action of chlorine p6 on human colon carcinoma cells. Results showed that chlorine p6 mediated photodynamic action induces apoptotic cell death when extracellular ph is 7.4 whereas cell death mostly occurs by necrosis when extracellular ph is 6.5. This shows that increased accumulation of photosensitizer occurring at lower ph may not be the reason for inhibition of apoptosis but PDT induced photodamage to membrane and alteration in ion homeostasis may be important in determining the mode of cell death.

N Vivacqua Gomes (2005)[44] studied the presence of *E-Faecalis* after root canal treatment in single or multiple visit and in conclusion neither single or multiple visit root canal treatment eliminated *E-Faecalis* completely from dentinal tubules. Upto 60 days after root filling *E-Faecalis* remain viable inside dentinal tubules.

Michael R Hablin et al (2005)[10] compared the interaction of three antimicrobial photosensitizer rose bengal(RB), toludene blue(TBO), and a polyl L lysine chlorine conjugate with a representative three classes of pathogens, *E- coli, staphylococcous aureus, candida albicans* .They compared fluence –dependendent cell survival after illumination with appropriate wavelength of light before and after extracellular dye has been washed out and used ten fold dilution of cell concentration.pLce6 was overall the most powerful photosensitizer .The overall order of susceptibility was *S.aureus > E.coli >albicans* and concluded that the number and mass of the cells complete both for available dye binding and for extracellularly generated deactive oxygen species.

Ziviluksiene et al (2005)[28] reviewed that photosensitization has been really effective against different microorganism such as drug resistant bacteria, yeast, virus and parasites and can be inactivated by this method.So photosensitization

phenomena can open a new and interesting avenues for the development of novel effective and ecologically friendly antimicrobial therapy.

Farzane Aghahosseini et al (2006)[1] reported that photodynamic therapy was used as a possible alternative method in treatment of lichen planus.Two patients with five oral lichen planus lesion was treated with topical PDT mediated by methylene blue .Patients were followed upon sessions 3,7,15 days and 1-9 months after PDT. Clinical improvement was achieved in 4 lesions.2 lesions showed complete remissions and other 2 had about 50% clinical improvement after a single session of PDT.No response was detected in one lesion and concluded that MB-PDT seemed to be an effective alternate treatment for control of OLP.

E Ercan et al (2006)[14] investigated the microorganisms isolated from necrotic pulp tissue and from failed endodontic treatments in infected dental root canals .In conclusion

prevalence of bacteria and fungi found from the root canal is the prevelance of microbial genera in primary and secondary endodontic infection. *Peptostreptococcous spp* was the predominantly isolated microbial genera followed by *streptococcous species ,porphyromonas spp, E.faecalis, staphylococcous, prevotella, lactilobacillus, actinomyces, candida albicans, fusobacterium spp.*

Aguinaldo Silva Garcz et al (2006)[17] investigated the action of the red laser associated with the photosensitizer on the reduction of *E-faecalis* in dental root canal. Results showed that photosensitizer alone do not have any antibacterial action.Laser photosensitization resulted in reduction of 99.2% of significantly higher reduction than NaOCl .So it was concluded that laser photosensitization was effective for reducing *E-Faecalis* in the root canal and could be a an adjuvant to endodontic treatment.

Alok Dube et al (2006)[12] studied pharmacokinetics and tissue response to PDT using chlorine p6 in hamster

cheek pouch model . It was observed that PDT of small tumors at 4 hours after IP injection of ClP6 resulted in complete tumor necrosis while PDT of large tumors receiving ClP6 topically causes necrosis in 300-800 µm superficial region of the tumor. It was observed that small tumors disappeared completely leaving no scar tissue ,while large tumor had significant reduction in tumor size, so the use of chlorine p6 for PDT of oral cancer is suggested.

SJ Bonsor (2006)[7] carried out a randomized trial in general dental practice to determine the microbiological effect of PDT as an adjuvant to normal root canal disinfection. Of 30 canals 10 canals were negative to culture ,these were either one of the canals in multirooted teeth were others were infected or were a retreatment with polyantibiotic paste had been applied to hyperaemic vital tissue . 16 of the remaining were negative to culture after the PAD process and concluded that PAD system offers a means of destroying bacteria

remaining after using conventional irrigants in endodontic therapy.

JA Williams (2006) [48] studied the antibacterial action of PAD on endodontic bacteria in planktonic suspension and root canal .Four bacteria, *fusobacterium nucleatum* , *peptostreptococcous microbes* ,*prevotella intermedia* ,*and streptococcus intermedia* were used. Results showed that in suspension , reduction in bacteria were highly significant for light/tolonium chloride combination compared with light or tolonium chloride alone .Maximum mean log reduction of 1.14 for .P intermedia ,2.48 for P micros , 2.81 for *F-nucleatum* and 6.73 for *S-intermedius* at 4.8 joules .In conclusion PAD killed endodontic bacteria at statistically significant levels compared to controls and kills varied with bacterial species.

Nikolaos Soukos et al (2006)[42] investigated the effects of PDT on endodontic planktonic phase as well as on *E-Faecalis* biofilms in experimentally infected root canal of

extracted tooth. Strains of microorganisms were sensitized with MB (25 µg/ml) for 5 minutes followed by exposure to red light of 665 nm with an energy fluence of 30 J/cm2 . Methylene blue fully eliminated all bacterial species with the exception of *E-Faecalis* (63%) killing . The same concentration of methylene blue in combination with red light was able to eliminate 97% of *E-Faecalis* biofilm bacteria in root canals .They concluded that PDT may be developed as an adjuvant procedure to kill residual bacteria in the root canal system after standard endodontic treatment.

L Bergmans et al studied (2006)[5] the role of Nd:YAG laser in root canal disinfection along with a minimally invasive treatment concept. Resulting CFU's of E-Faecalis were were associated with the observation of bacterial cell structural changes using conventional scanning electron microscopy on inoculated dentin surface,following indirect and direct Nd:YAG laser irradiation.Results of the study showed that Nd:YAG irradiation of root canals infected with E-Faecalis

resulted in significant reduction of the bacterial load(99.7%),but not complete sterilization. It was concluded that Nd;YAG laser irradiation is not an alternative but a possible supplement to existing protocols for canal disinfection and even direct exposure could not eradicate endodontic pathogens that grow in biofilm.

A.U. Eldeniz et al (2007)[13] compared the efficacy of a standard NaOCl irrigation procedure with that of Er,Cr:YSGG laser irradiation in contaminated root canals having small and large apical foramina.It was concluded that in teeth with straight roots Er,Cr:YSGG laser reduced the viable microbial population in root canal with small and large apical foramina but did not eradicate all bacteria. 3% NaOCl inhibited the growth of *E-Faecalis* and effectively sterilized all root canals.

Aguinaldo Garcez et al (2007)[18] compared the effectiveness of antimicrobial PDT, standard endodontic treatment and the combined treatment to eliminate bacterial biofilms present in

infected root canals .Results showed that endodontic therapy alone reduced the bacterial bioluminesence by 90% while PDT alone reduced the bacterial load by 95% and the combination treatment reduced the bacterial load by 98%. It was concluded that antimicrobial PDT may have a role to play in optimized endodontic therapy.

LWN Van der Sluis et al(2007)[43] reviewed that use of passive ultrasonic irrigation of the rootcanals and concluded that PUI appears as an adjunctive treatment for cleaning the root canal system and PUI is more effective than syringe irrigation and stated that more research is needed to clarify the underlying physical mechanism through which PUI exerts its efficacy.

Ulrich Schoop et al (2007)[32] used Er,Cr:YSGG laser at 2 power settings to see the efficacy against 2 bacteria inoculated in root canals and subjected them to a quantitive microbiological evaluation.they also used SEM to assess morphological changes in endodontically processes and laser-

irradiated root canal walls. They also measured temperature increases on the root surface to determine possible thermal side effects. Bacterial evaluation revealed a disinfecting effect in root samples that was dependant on the out power but not specific for the bacterial species investigated.SEM showed the removal of the smear layer from the root canal walls and the exposure of dentinal tubules and the temperature rise during irradiation was moderate when standardized power settings were used.

Wanda Gordon et al(2007)[20] investigated the Er,Cr:YSGG laser with radial emitting tips to disinfect E-Faecalis infected dentin. Authors found that bacterial recovery decreased when laser irradiation duration or power increased.a greater degree of disinfection was achieved with a 120 s application of laser than with hypochlorite treatment.In conclusion they found that a 99.7% reduction in bacterial counts could be obtained using the laser.

Ali Mahmoudpour et al(2007)[30] designed a study to survey the incidence of *E-Faecalis* infection in symptomatic and asymptomatic root canals of necrotic teeth using PCR and to isolate the bacterium for further screening. Results showed that 4 isolates were obtained from asymptomatic cases of chronic apical periodontitis and other 2 were associated with phoenix abscess and acute apical abscess respectively. No *E-Faecalis* infection were found in 5 patients with acute apical periodontitis or in 9 with chronic suppurative periodontitis .So the results indicate there is no significant difference in the incidence of *E-Faecalis* between symptomatic and asymptomatic necrotic dental root canals.

K Konopka et al (2007)[24] reviewed that application of PDT in dentistry is growing rapidly in the treatment of oral cancer, bacterial and fungal infection therapy, and photodynamic diagnosis of the malignant transformation of oral lesions, PDT has shown potential in treatment of oral leukoplakia, oral lichen planus ,and head

and neck cancer. PACT has been efficacious in treatment of bacterial, fungal and parasitic and viral infections. PDT also presents a novel therapeutic approach in management of oral biofilms.

Tim Maisch et al(2007)[31] to elucidate the oxidative process that occur during killing of bacteria, *staphylococcus aures* was incubated with a standard photosensitizer, and the generation and decay of singlet oxygen was detected directly by its luminescence at 1,270nm.At low bacterial concentrations, time-resolved luminescence of singlet oxygen showed a decay time of 6 ± 2µs,which is an intermediate time for singlet oxygen decay in phospholipids of membranes and in the surrounding water. Obviously,at low bacterial concentration, singlet oxygen had seems to be generated in the outer call walls areas or in the adjacent cytoplasmic membranes of S aures.In addition detection of singlet oxygen can be used as a sensor of intracellular oxygen concentration.When singlet

oxygen luminescence was measured at higher bacterial concentration, the decay time increased significantly, upto ~ 40µs,because of oxygen depletion at these concentrations.This observation is an important indicator that oxygen supply is a crucial factor in the efficacy of photodynamic inactivation of bacteria, and will be of particular significance should this approach be used against multiresistant bacteria.

L Bergmans et al(2008)[6] aimed to test the hypothesis that PAD has a bacteriocidal effect on pathogens inoculated in root canals, with emphasis on biofilm formation/destruction.It was concluded that PAD is not an alternative but a possible supplement to the existing protocols for root canal disinfection. As the interaction between light and associated dye provides a broad spectrum effect.

Mrinalini Sharma et al(2008)[36] investigated the effect of the photodynamic action of TBO and laser simultaneously.

The effect was found to be light dose dependant. Confocal microscopy suggested that damage to bacterial cell membranes in photodynamically treated biofilms .In addition SEM provided direct evidence for the distruption of biofilm structure and a decrease in cell number in PDT treated biofilm.Results suggests that photodynamic treatment may be a useful approach in the inactivation of staphylococcal biofilm adhering to solid surfaces of medical implant

Biplab et al(2008)[8] studied the photodynamic efficacy of chlorine p6 at ph 5,6,and 7.6 in aqueous and liquid environment.Increase chlorine p6 medited PDT bleaching of NN –dimethyl – 4- nitroso aniline ,a measure of singlet oxygen yield, was obtained at a higher ph. Rate of PAD bleaching of RNO was also higher at higher ph and rate decreased in lowering in ph of irradiated solution .PDT oxygenation of tryptophan was found to be higher at higher pH.At lower pH chlorine p6 mediated PDT malon dialdehyde and lipid hydroperoxide formation in egg lecithin liposome

was higher. At higher pH chlorine p6 was found to be photodynamically more effective in aqueous environment whereas at lower ph chlorine p6 was more effective in hydrophobic ph.

Fonesca et al (2008)[16] investigated the effect of PDT on endogenic pathogens by evaluating the decrease in number of E -Faecalis colony in canals of extracted human teeth. Results showed that mean decrease in CFU was 99.9 % in the test group where as in control group an increase of 2.6% was observed.it Is concluded that PDT was effective as a bactericidal agent in *E.faecalis* contaminated root canal.

Jacob Lee Fimple et al (2008)[15] investigated the PDT of methylene blue on multispecies root canal biofilms comprising *actinomyces Israeli , F-Nucleatum, P-Gingivalis* and *prevotella* intermedia.PDT achieved 80% reduction of CFU counts and concluded that PDT can be an effective adjuvant to standard endodontic antimicrobial treatment when PDT paramaters are optimized.

Aguinaldo Silva Garcez et al (2008)[19] analyzed the antimicrobial effect of PDT in association with endodontic treatment. Results suggest that PDT added to endodontic treatment leads to an enhanced decrease of bacterial load and may be an appropriate approach for treatment of oral infections.

Pilar Baca et al (2009)[4] conducted a double blinded randomized clinical trials in 68 subjects .21 subjects with 60 root caries lesions and 25 with 65 lesions in the cervitec and placebo groups respectively.varnish were applied twice in the week, 1 month later and every 3 months.Results showed that clinical evolution was significantly different in cervitec group as opposed to the placebo group in terms of width, height, colour and texture.

B Dickers et al (2009)[11] studied to determine whether it is safe to use PAD during root canal infection without heating periodontal tissue. Temperatures were recorded on the

external root surface. Results showed that after 150 sec of PAD irradiation, average temperature rise was .16 ± .08 ºc . All values were lower than the 7ºc safety level for periodontal injury.It was concluded that regarding the temperature increase ,use of PAD in root canal could be harmless for periodontal tissue.

MATERIALS

1. Extracted upper cental incisors.
2. Syringe [Hindustan Syringes & Medical Devices LTD, India]
3. Enterococcus faecalis [ATCC 29212]
4. Absorbent paper points [Dentsply Maillefer, Switzerland]
5. Hypochlorite 2.5% [Prime Dental Products PVT LTD, India]
6. Normal saline [Nirlife Health Care, Nirma Products, India]
7. Phosphate buffered saline.
8. Tryptic soya bean growth medium [Becton,Dickinson,and Co,Sparks,MD].
9. Diamond discs.
10. Agar [Sigma Aldrich ,Germany]
11. Micro pipette [Eppendorf, Germany]
12. Conical flasks.
13. Loops.
14. Plastic spreaders.
15. EDTA 17%.[Tulsa, OK, USA]
16. Chlorine P6 dye. [porphyrin products,Logan,UT]
17. Petridishes.

ARMAMENTARIUM

18. Hand piece [PAN AIR, NSK, Japan]
19. Light curing unit [3M ESPE ,Seefeld,Germany]
20. Incubator.[biotechniques,India]
21. Access opening bur [Mani Inc, Japan]
22. K-Files [Mani Inc, Japan]
23. Micromotor with contra angled hand piece. (NSK,Japan)
24. Gates Glidden Drills [Dentsply Maillefer,Tulsa,OK].

SPECIAL EQUIPMENTS

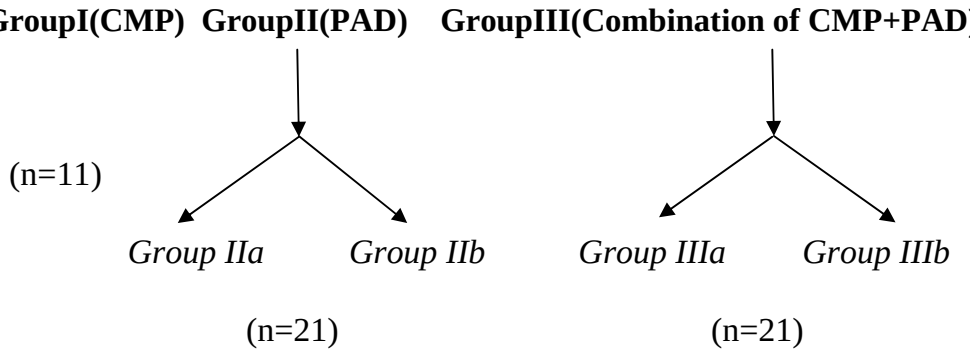
25. Diode laser unit(660nm) with fiber coupled[SDL-660-LM-X, Perfect Laser (Wuhan) Co, Ltd].
26. Autoclave [Uniclave C-79, Confident Dental Equipment LTD, India]
27. Laminar flow chamber.[biotechniques,India].
28. Cintra 20-uv-visible spectrometer.[GBC scientific instruments,Germany]
29. G-Box gel doc and analysis.[Chemi HR-16-XT-16,syngene,Cambridge,U.S.A.]

METHODOLOGY

53 freshly extracted human maxillary incisors with straight canals, extracted for periodontal reasons, were collected. The teeth were cleaned using an ultrasonic scaler and were stored in solution of 0.5% chloramines in water at 4°C until employed in the experiment . The teeth were decoronated using diamond disk and roots were standardized to a length of approximately 14 mm. Patency of apical foramen was established by inserting a size 15 K file. File measurement was taken at the point where the size 15 K file became visible at apical foramen and 0.5mm was subtracted to set working length. The instrumentation sequence consisted of Gates Glidden drills 4, 3 and 2 for coronal 4mm preparation, followed by an apical preparation till MAF 40 size with K-files, using hybrid technique and the root canals were irrigated and cleaned with 5ml of 2.5% sodium hypochlorite and 5ml of 17% EDTA solution between each endodontic file and final flush was done with 0.9% w/v normal saline.

Grouping:

Teeth were divided into 3 Groups, Group I consisted of (n=11) samples, Group II(n=21) samples and group III(n=21) samples, Group II and Group III were further divided into 2 subgroups, Group IIa , IIb and Group IIIa , IIIb.



Group I (Conventional Endodontic Treatment of root canal)

In this group the root canals were irrigated with 17% EDTA for 2 min followed by irrigation with saline to remove the smear layer. The apical foramen was subsequently closed with composite resin restoration. The external root surface of all the teeth were sealed with two layers of nail polish to avoid environmental contamination. All the prepared samples were mounted in small vials and autoclaved at 121º C,15 lbs pressure for 15 minutes to

ensure complete sterilization of root canals before inoculation with microorganism, *E-Faecalis*.

Bacterial growth

E-Faecalis was grown in Tryptic Soya Bean broth by overnight culturing in an incubater at 37º C to form a stationary growth phase.

Inoculation of bacteria

Bacterial growth was confirmed using microscope.

All samples were inoculated with 10 µl of the broth containing known number of *E-Faecalis* (2.5×10^4/ ml) using a micropipette (15µl).

All the samples were incubated for 24 hours at 37 º C.

Treatment

Conventional endodontic treatment was performed till MAF size 60 using K-files. The canals were irrigated with 10 ml of 2.5 % NaOCl and 17% EDTA solution 10 ml alternatively between each file using a 28 gauge needle and syringe. The final flush was done with 0.9% w/v normal saline. To prevent external contamination of root surface by overflowing irrigant, the teeth were held inverted during the irrigation procedure.

Group II (Photo Activated Disinfection of root canal)

In Group II all the teeth were further prepared apically till MAF 60 size using K-file. Canals were irrigated with hypochlorite (2.5%) (5ml) and saline (5ml) alternatively between each file using a 28 gauge needle. The root canals were irrigated with 17% EDTA for two min followed by irrigation with saline to remove the smear layer. The apical foramen was subsequently closed with composite resin restoration. The external root surface of all the teeth were sealed with two layers of nail polish to avoid environmental contamination. All the prepared samples were mounted in small vials and autoclaved at 121º C,15lbs pressure for 15 minutes to ensure complete sterilization of root canals before inoculation with microorganism, *E-Faecalis*.

Bacterial growth

E-Faecalis was grown in Tryptic Soya Bean broth by overnight culturing in an incubater at 37º C to form a stationary growth phase.

Inoculation of bacteria

Bacterial growth was confirmed using microscope.

All samples were inoculated with 10 µl of the broth containing known number of *E-Faecalis* (2.5×10^4/ ml) using a micropipette (15µl).

All the samples were incubated for 24 hours at 37 º C.

Treatments

In Group II, the teeth were further divided into two subgroups, Group IIa and Group IIb. In both the groups the root canals were dried using paper points to remove any contents left inside the root canals. The canals were filled with 10 µM of Photosensitizer(PS) solution of chlorine p6. The root canals were again dried with paper points after 10 min.

Group IIa

In Group IIa disinfection of root canal was performed with a 600µm, 670 nm fiber coupled diode laser for 4 minutes which delivered a total power of 65 mW at the tip. The fiber was initially placed in 2mm short of apex and moved gradually towards the middle and cervical third of canal to impart thorough

disinfection of the canal. These movements were repeated approximately 6 times per minute.

Group IIb

In Group IIb disinfection of root canal was performed with a 600µm, 670 nm fiber coupled diode laser as above but for a time period of 2 minutes only.

Group III (Combination of endodontic treatment with PAD)

In this group the root canals were irrigated with 17% EDTA for 2 min followed by irrigation with saline to remove the smear layer. The apical foramen was subsequently closed with composite resin restoration. The external root surface of all the teeth were sealed with two layers of nail polish to avoid environmental contamination. All the prepared samples were mounted in small vials and autoclaved at 121º C,15lbs pressure for 15, minutes to ensure complete sterilization of root canals before inoculation with microorganism, *E-Faecalis*.

Bacterial growth

E-Faecalis was grown in Tryptic Soya Bean broth by overnight culturing in an incubater at 37º C to form a stationary growth phase.

Inoculation of bacteria

Bacterial growth was confirmed using microscope.

All samples were inoculated with 10 µl of the broth containing known number of *E-Faecalis* (2.5×10^4/ ml) using a micropipette (15µl).

All the samples were incubated for 24 hours at 37 º C.

Treatment

Conventional endodontic treatment was performed till MAF size 60 using K-files. The canals were irrigated with 10 ml of 2.5 % NaOCl and 17% EDTA solution 10 ml alternatively between each file using a 28 gauge needle and syringe. The final flush was done with 0.9%w/v normal saline. To prevent external contamination of root surface by overflowing irrigant, the teeth were held inverted during the irrigation procedure.

The teeth were again divided into Group IIIa and Group IIIb. Canals were dried using paper points to remove any irrigant left inside the

root canals.The canals were filled with 10 µM solution of photosensitizer(PS), chlorine P6 and the root canals were again dried with paper points after 10 min.

Group IIIa

In Group IIIa disinfection of root canal was performed with a 600 µm, 670 nm fiber coupled diode laser for 4 minutes which delivered at total power of 65 mW out of fiber. The fiber was initially placed in 2mm short of apex and moved gradually towards the middle and cervical third of canal to impart thorough disinfection of the canal. These movements were repeated approximately 6 times per minute.

Group IIIb

In Group IIIb disinfection was performed with a 600 µm, 670 nm fiber coupled diode laser as above but for a time period of 2 minutes only.

Control group

One tooth from each group was taken as the positive control where no treatment was done after inoculation of bacteria.

Bacterial evaluation

Root canals were filled with Phosphate Buffered Saline (PBS) and gently filed in a circumferential way using 25 size file to working length. The contents of root canals were aspirated using a syringe into vials and serially diluted with PBS. 100 microlitre of each dilution was plated in culture plates containing Tryptone Soya Bean (TSB) agar medium. The plates were incubated at 37ºC for 24 hours under anaerobic conditions. CFU's were counted after 24 hrs in each group.

The cell death or the percentage of bacterial killing was calculated from the CFU's counted in the culture plates after 24 hours.

MATERIALS

1. Extracted upper cental incisors.
2. Syringe [Hindustan Syringes & Medical Devices LTD, India]
3. Enterococcus faecalis [ATCC 29212]
4. Absorbent paper points [Dentsply Maillefer, Switzerland]
5. Hypochlorite 2.5% [Prime Dental Products PVT LTD, India]
6. Normal saline [Nirlife Health Care, Nirma Products, India]
7. Phosphate buffered saline.
8. Tryptic soya bean growth medium [Becton,Dickinson,and Co,Sparks,MD].
9. Diamond discs.
10. Agar [Sigma Aldrich ,Germany]
11. Micro pipette [Eppendorf, Germany]
12. Conical flasks.
13. Loops.
14. Plastic spreaders.
15. EDTA 17%.[Tulsa, OK, USA]
16. Chlorine P6 dye. [porphyrin products,Logan,UT]
17. Petridishes.

ARMAMENTARIUM

18. Hand piece [PAN AIR, NSK, Japan]
19. Light curing unit [3M ESPE ,Seefeld,Germany]
20. Incubator.[biotechniques,India]
21. Access opening bur [Mani Inc, Japan]
22. K-Files [Mani Inc, Japan]
23. Micromotor with contra angled hand piece. (NSK,Japan)
24. Gates Glidden Drills [Dentsply Maillefer,Tulsa,OK].

SPECIAL EQUIPMENTS

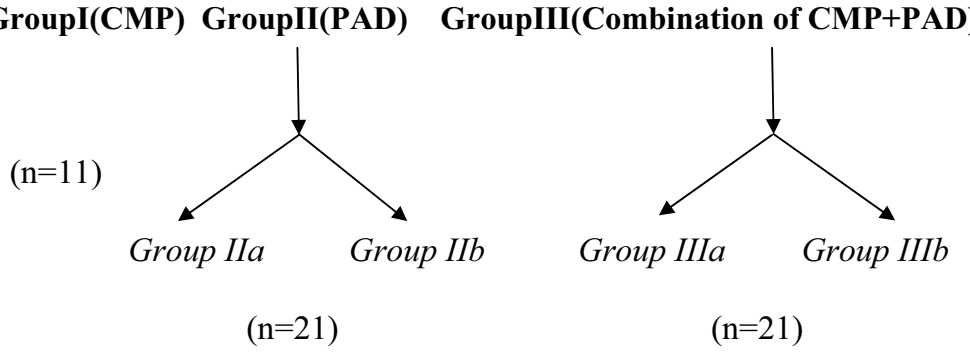
25. Diode laser unit(660nm) with fiber coupled[SDL-660-LM-X, Perfect Laser (Wuhan) Co, Ltd].
26. Autoclave [Uniclave C-79, Confident Dental Equipment LTD, India]
27. Laminar flow chamber.[biotechniques,India].
28. Cintra 20-uv-visible spectrometer.[GBC scientific instruments,Germany]
29. G-Box gel doc and analysis.[Chemi HR-16-XT-16,syngene,Cambridge,U.S.A.]

METHODOLOGY

53 freshly extracted human maxillary incisors with straight canals, extracted for periodontal reasons, were collected. The teeth were cleaned using an ultrasonic scaler and were stored in solution of 0.5% chloramines in water at 4°C until employed in the experiment . The teeth were decoronated using diamond disk and roots were standardized to a length of approximately 14 mm. Patency of apical foramen was established by inserting a size 15 K file. File measurement was taken at the point where the size 15 K file became visible at apical foramen and 0.5mm was subtracted to set working length. The instrumentation sequence consisted of Gates Glidden drills 4, 3 and 2 for coronal 4mm preparation, followed by an apical preparation till MAF 40 size with K-files, using hybrid technique and the root canals were irrigated and cleaned with 5ml of 2.5% sodium hypochlorite and 5ml of 17% EDTA solution between each endodontic file and final flush was done with 0.9% w/v normal saline.

Grouping:

Teeth were divided into 3 Groups, Group I consisted of (n=11) samples, Group II(n=21) samples and group III(n=21) samples, Group II and Group III were further divided into 2 subgroups, Group IIa , IIb and Group IIIa , IIIb.



Group I (Conventional Endodontic Treatment of root canal)

In this group the root canals were irrigated with 17% EDTA for 2 min followed by irrigation with saline to remove the smear layer. The apical foramen was subsequently closed with composite resin restoration. The external root surface of all the teeth were sealed with two layers of nail polish to avoid environmental contamination. All the prepared samples were mounted in small vials and autoclaved at 121º C,15 lbs pressure for 15 minutes to

ensure complete sterilization of root canals before inoculation with microorganism, *E-Faecalis*.

Bacterial growth

E-Faecalis was grown in Tryptic Soya Bean broth by overnight culturing in an incubater at 37º C to form a stationary growth phase.

Inoculation of bacteria

Bacterial growth was confirmed using microscope.

All samples were inoculated with 10 µl of the broth containing known number of *E-Faecalis* (2.5×10^4/ ml) using a micropipette (15µl).

All the samples were incubated for 24 hours at 37 º C.

Treatment

Conventional endodontic treatment was performed till MAF size 60 using K-files. The canals were irrigated with 10 ml of 2.5 % NaOCl and 17% EDTA solution 10 ml alternatively between each file using a 28 gauge needle and syringe. The final flush was done with 0.9% w/v normal saline. To prevent external contamination of root surface by overflowing irrigant, the teeth were held inverted during the irrigation procedure.

Group II (Photo Activated Disinfection of root canal)

In Group II all the teeth were further prepared apically till MAF 60 size using K-file. Canals were irrigated with hypochlorite (2.5%) (5ml) and saline (5ml) alternatively between each file using a 28 gauge needle. The root canals were irrigated with 17% EDTA for two min followed by irrigation with saline to remove the smear layer. The apical foramen was subsequently closed with composite resin restoration. The external root surface of all the teeth were sealed with two layers of nail polish to avoid environmental contamination. All the prepared samples were mounted in small vials and autoclaved at 121º C,15lbs pressure for 15 minutes to ensure complete sterilization of root canals before inoculation with microorganism, *E-Faecalis*.

Bacterial growth

E-Faecalis was grown in Tryptic Soya Bean broth by overnight culturing in an incubater at 37º C to form a stationary growth phase.

Inoculation of bacteria

Bacterial growth was confirmed using microscope.

All samples were inoculated with 10 µl of the broth containing known number of *E-Faecalis* (2.5×10^4/ ml) using a micropipette (15µl).

All the samples were incubated for 24 hours at 37 º C.

Treatments

In Group II, the teeth were further divided into two subgroups, Group IIa and Group IIb. In both the groups the root canals were dried using paper points to remove any contents left inside the root canals. The canals were filled with 10 µM of Photosensitizer(PS) solution of chlorine p6. The root canals were again dried with paper points after 10 min.

Group IIa

In Group IIa disinfection of root canal was performed with a 600µm, 670 nm fiber coupled diode laser for 4 minutes which delivered a total power of 65 mW at the tip. The fiber was initially placed in 2mm short of apex and moved gradually towards the middle and cervical third of canal to impart thorough

disinfection of the canal. These movements were repeated approximately 6 times per minute.

Group IIb

In Group IIb disinfection of root canal was performed with a 600µm, 670 nm fiber coupled diode laser as above but for a time period of 2 minutes only.

Group III (Combination of endodontic treatment with PAD)

In this group the root canals were irrigated with 17% EDTA for 2 min followed by irrigation with saline to remove the smear layer. The apical foramen was subsequently closed with composite resin restoration. The external root surface of all the teeth were sealed with two layers of nail polish to avoid environmental contamination. All the prepared samples were mounted in small vials and autoclaved at 121º C,15lbs pressure for 15, minutes to ensure complete sterilization of root canals before inoculation with microorganism, *E-Faecalis*.

Bacterial growth

E-Faecalis was grown in Tryptic Soya Bean broth by overnight culturing in an incubater at 37º C to form a stationary growth phase.

Inoculation of bacteria

Bacterial growth was confirmed using microscope.

All samples were inoculated with 10 µl of the broth containing known number of *E-Faecalis* (2.5×10^4/ ml) using a micropipette (15µl).

All the samples were incubated for 24 hours at 37 º C.

Treatment

Conventional endodontic treatment was performed till MAF size 60 using K-files. The canals were irrigated with 10 ml of 2.5 % NaOCl and 17% EDTA solution 10 ml alternatively between each file using a 28 gauge needle and syringe. The final flush was done with 0.9%w/v normal saline. To prevent external contamination of root surface by overflowing irrigant, the teeth were held inverted during the irrigation procedure.

The teeth were again divided into Group IIIa and Group IIIb. Canals were dried using paper points to remove any irrigant left inside the

root canals.The canals were filled with 10 µM solution of photosensitizer(PS), chlorine P6 and the root canals were again dried with paper points after 10 min.

Group IIIa

In Group IIIa disinfection of root canal was performed with a 600 µm, 670 nm fiber coupled diode laser for 4 minutes which delivered at total power of 65 mW out of fiber. The fiber was initially placed in 2mm short of apex and moved gradually towards the middle and cervical third of canal to impart thorough disinfection of the canal. These movements were repeated approximately 6 times per minute.

Group IIIb

In Group IIIb disinfection was performed with a 600 µm, 670 nm fiber coupled diode laser as above but for a time period of 2 minutes only.

Control group

One tooth from each group was taken as the positive control where no treatment was done after inoculation of bacteria.

Bacterial evaluation

Root canals were filled with Phosphate Buffered Saline (PBS) and gently filed in a circumferential way using 25 size file to working length. The contents of root canals were aspirated using a syringe into vials and serially diluted with PBS. 100 microlitre of each dilution was plated in culture plates containing Tryptone Soya Bean (TSB) agar medium. The plates were incubated at 37ºC for 24 hours under anaerobic conditions. CFU's were counted after 24 hrs in each group.

The cell death or the percentage of bacterial killing was calculated from the CFU's counted in the culture plates after 24 hours.

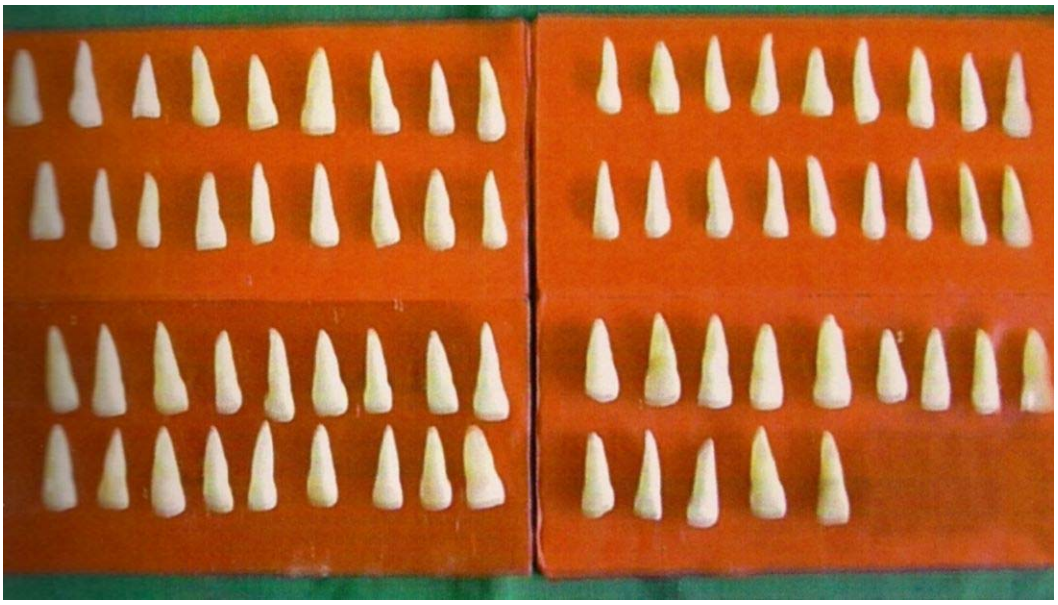


Fig 1: TOOTH SPECIMENS

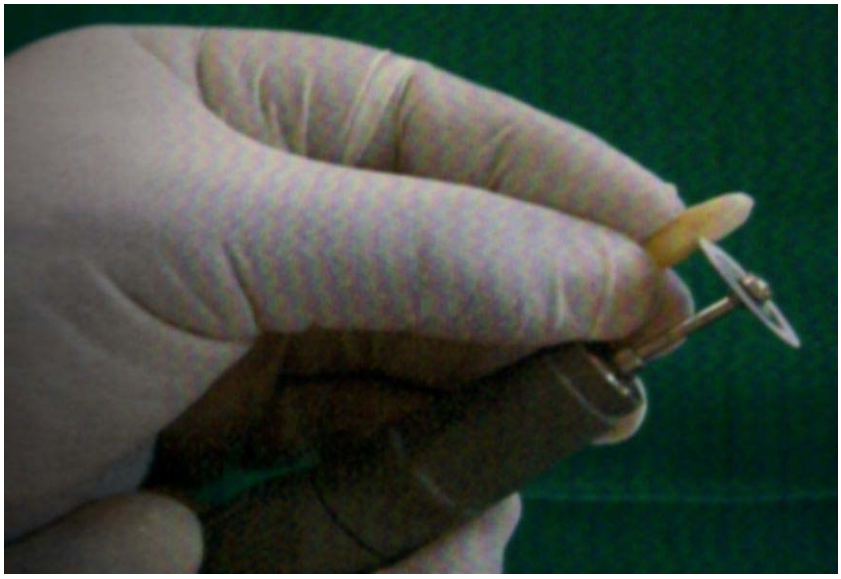


Fig 2: DECORONATION

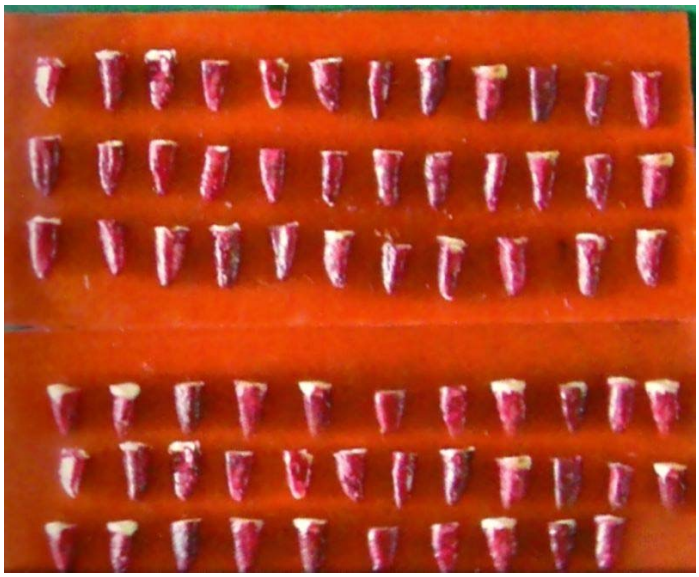


Fig 3: DECORONATED SAMPLES

Fig 4: ARMAMENTARIUM

Fig 5: MATERIALS FOR AUTOCLAVING

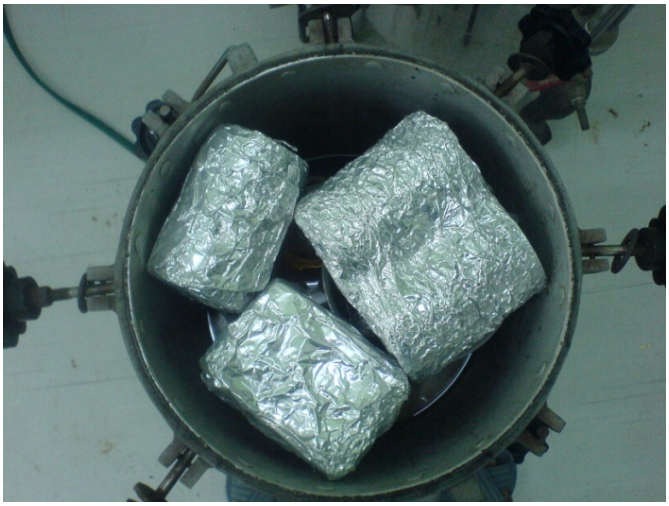


Fig 6: AUTOCLAVE

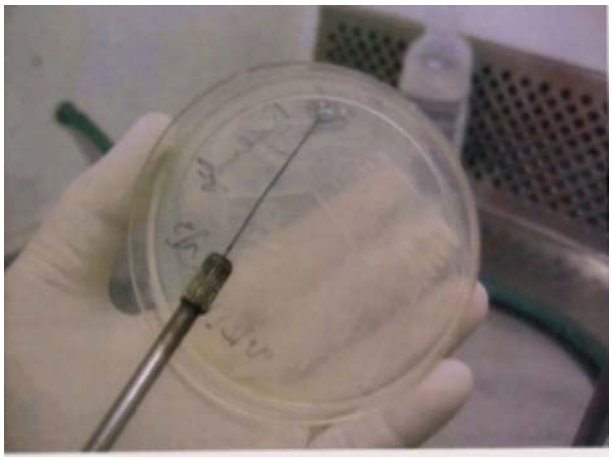


Fig 7:STREAKING OF E-FAECALIS

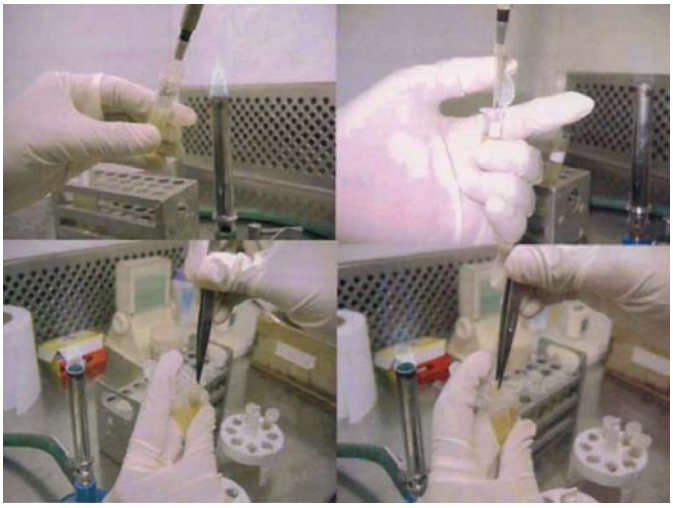


Fig 8: INFECTING THE TOOTH SAMPLES

Fig 9: INCUBATORS

Fig 10: LABORATORY SETUP

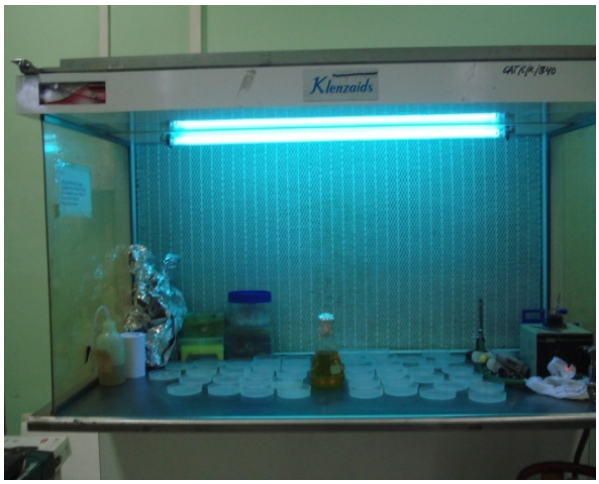


Fig 11: LAMINAR FLOW

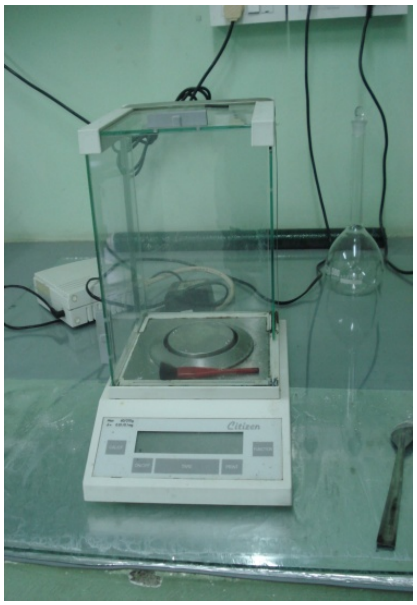


Fig 12: ELECTRONIC WEIGHING BALANCE

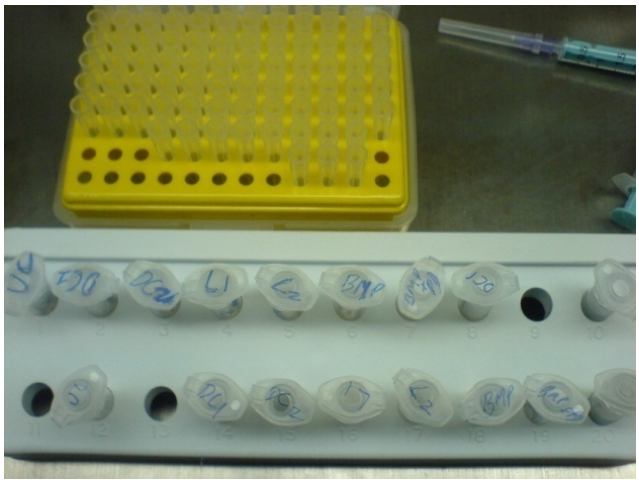


Fig 13: SAMPLES

Fig 14: POWER METER

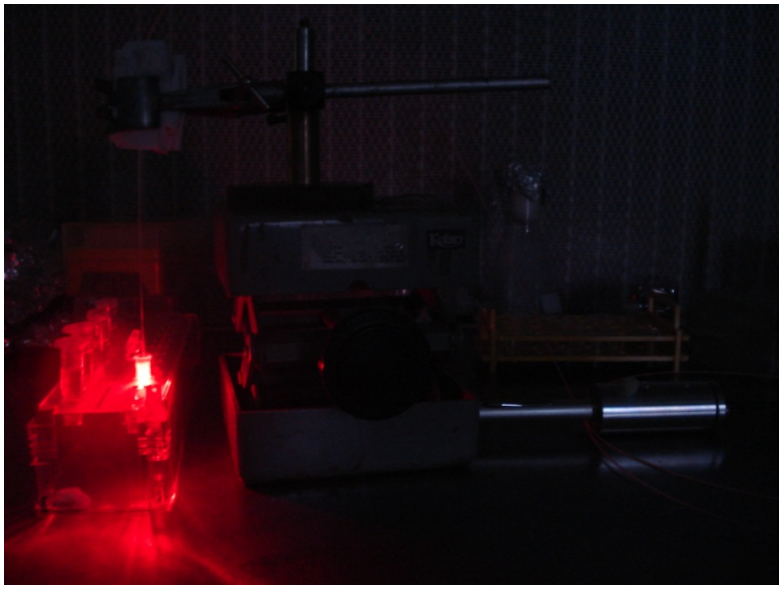


Fig 15: PHOTO-ACTIVATED DISINFECTION OF ROOT CANALS

Fig 16: EPPIDOFF PIPPETE

Fig 17: INCUBATION OF CULTURE PLATES

Fig 18: G-BOX-GEL DOCUMENTATION UNIT AND PLATE READER

Fig 19: CINTRA-20.LIGHT SPECTROMETER

Fig 20: CONTROL GROUP

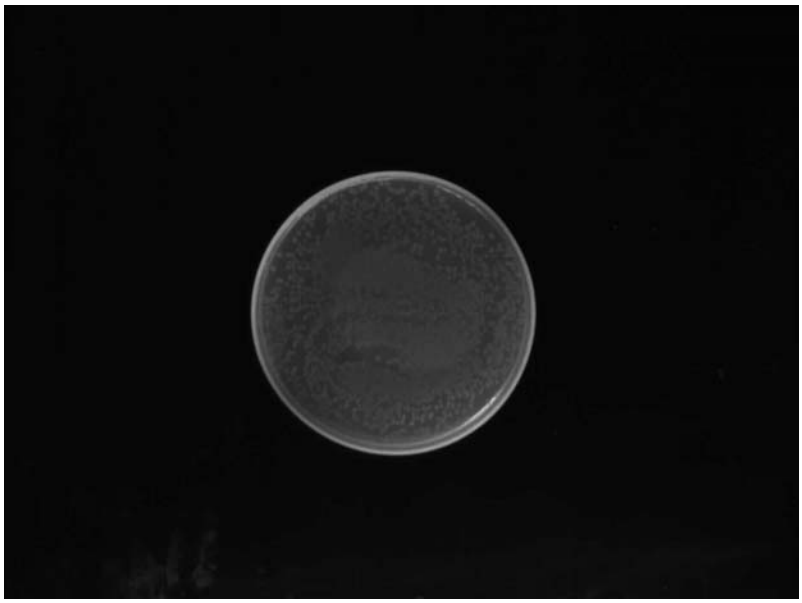


Fig 21: GROUP I (CMP)

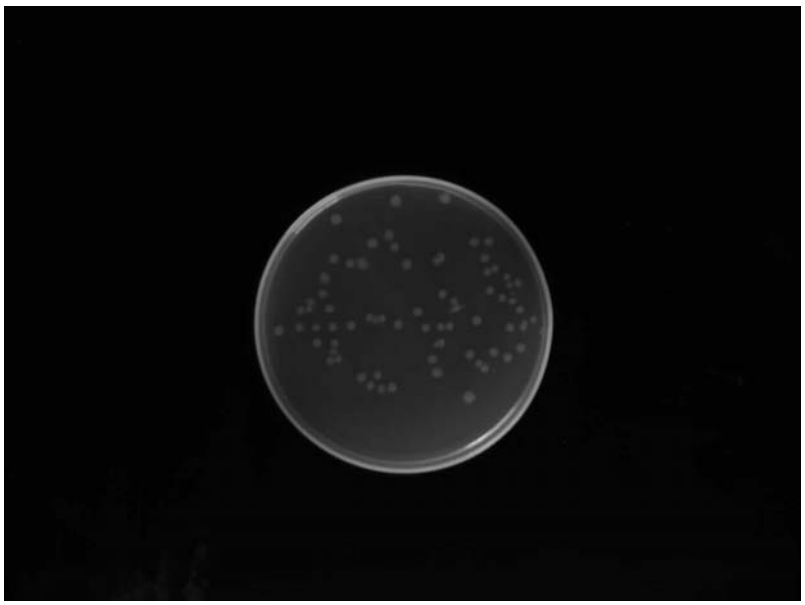


Fig 22: GROUP IIA (PAD 4 MIN)

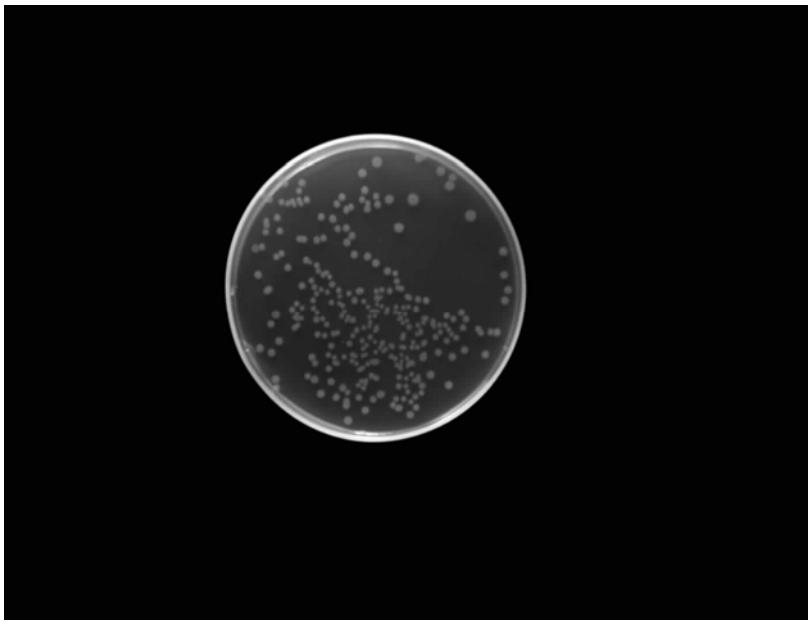


Fig 23: GROUP IIB (PAD 2MIN)

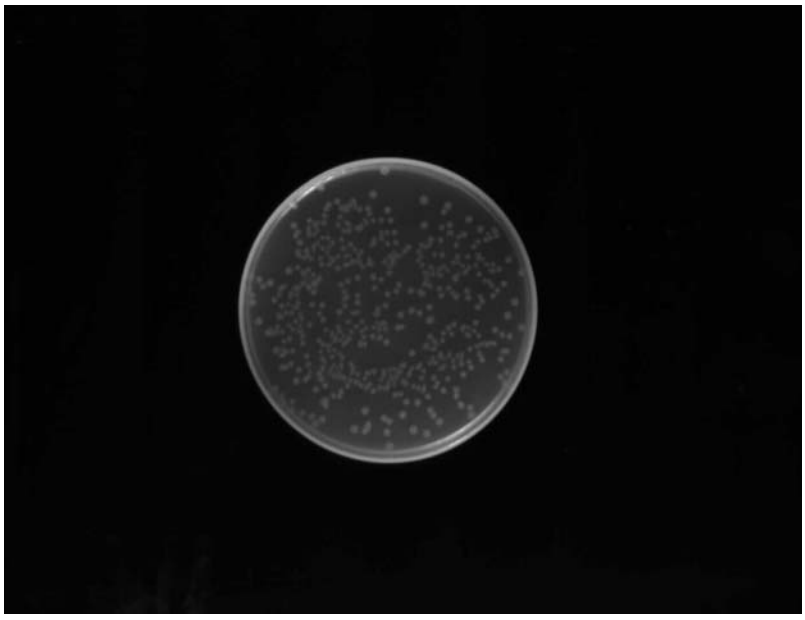


Fig 24: GROUP IIIA(CMP + PAD 4 MIN)

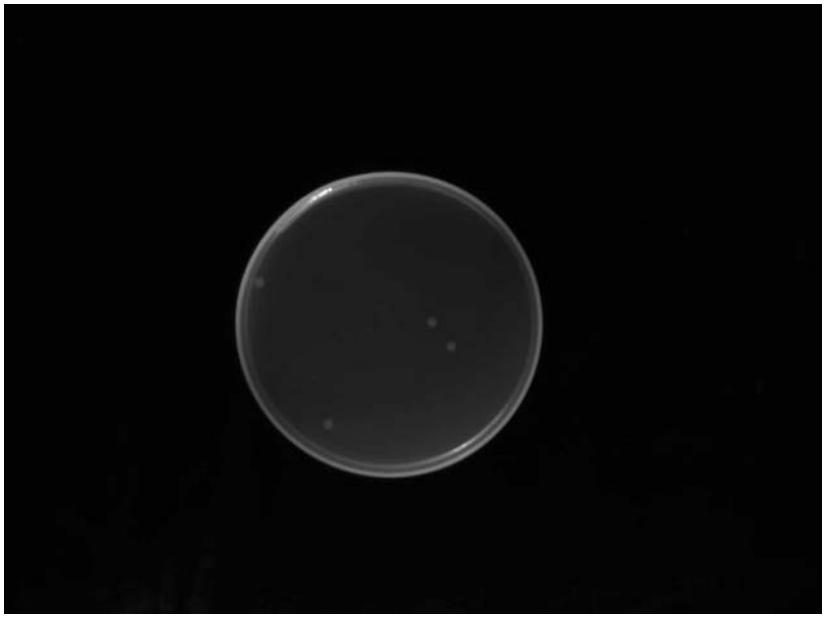
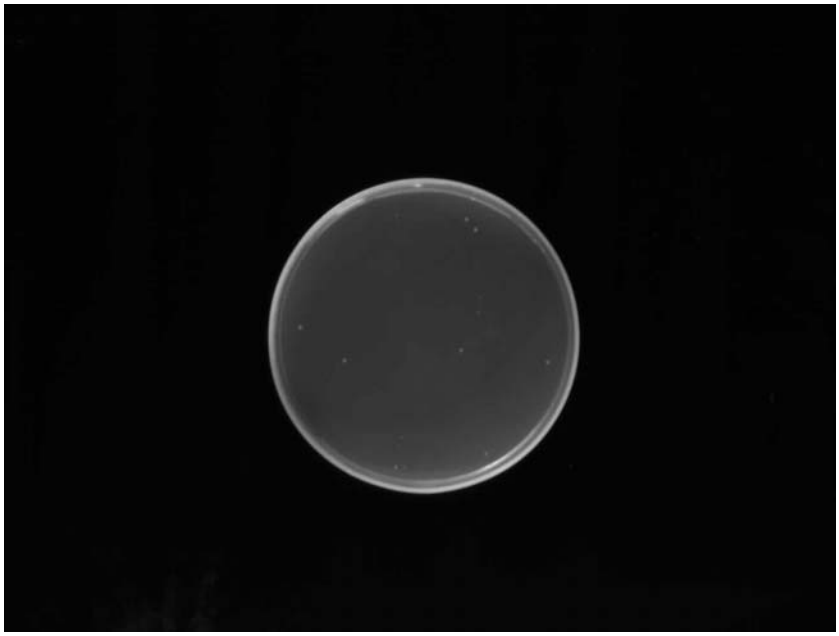


Fig 25: GROUP IIIB(CMP + PAD 2 MIN)



RESULTS

The surviving fraction and the cell death of each group calculated are represented in the following tables.(Table I,IIa,IIb,IIIa,IIIb)

The cell death was calculated from the CFU's counted in the plates after 24 hours.

$$\text{Surviving fraction (\%)} = \frac{\text{No of CFU's in the untreated control / ml}}{\text{No of CFU's in the treated group / ml}} \times 100$$

Cell death (%) = 100 – Surviving fraction.

The untreated control group(3 specimen) were used as a reference to calculate the surviving fraction and cell death.

Cell death denotes exactly the efficiency of treatment or the reduction of bacteria, in terms of percentage which gives a better representation of bacterial killing.

DATA COLLECTION:

Table I - Datas for Group I (CMP)

Sample No.	Surviving fraction (%)	Cell Death (%)
1	12.7	87.3
2	12.2	87.8
3	13.7	86.3
4	8.7	91.3
5	11.5	88.5
6	9.7	90.3
7	10.2	89.8
8	10.9	89.1
9	11.7	88.3
10	10.7	89.3
Mean ± SD	11.25 ± 1.48	88.80 ± 1.18

Table IIa - Datas for Group IIa (PDT 4 min)

Sample No.	Surviving fraction (%)	Cell Death (%)
1	21.2	78.8
2	20.7	79.3
3	18.9	81.1
4	13.7	86.3
5	22.3	77.7
6	16.1	83.9
7	19.1	80.9
8	15.3	84.7
9	12.1	87.9
10	13.2	86.8

Mean ± SD	17.25 ± 3.61	82.81 ± 3.62

Table IIb - Datas for group I1b (PDT 2 min)

Sample No.	Surviving fraction (%)	Cell Death (%)
1	23.1	76.9
2	18.5	81.5
3	24.7	75.3
4	22.8	77.2
5	19.2	80.8
6	16.9	83.1
7	22.4	77.6
8	18.1	81.9
9	23.5	76.5
10	18.8	81.2

Mean ± SD	20.80 ± 2.76	79.20 ± 2.76

Table IIIa - Datas for Group IIIa (CMP and PDT 4 min)

Sample No.	Surviving fraction (%)	Cell Death (%)
1	0	100
2	0.8	99.2
3	0.1	99.9
4	0	100
5	1	99
6	0.3	99.7
7	1	99
8	0.7	99.3
9	0.9	99.1
10	0.2	98.8
Mean ± SD	0.50 ± 0.42	99.50 ± 0.16

Table IIIb - Datas for Group IIIb (PDT and BMP 2 min)

Sample No.	Surviving fraction (%)	Cell Death (%)
1	1.7	98.3
2	0.3	99.7
3	1.7	98.3
4	1.1	98.9
5	1.9	98.1
6	0.5	99.5
7	1.1	98.9
8	1.6	98.4
9	0.5	99.5
10	0.7	99.3
Mean ± SD	1.11 ± 0.59	98.89 ± 0.59

STATISTICAL ANALYSIS -

The results of the present study were subjected to statistical analysis to interpret the significant difference among various treatment groups, and between the subgroups. One way ANOVA, post HOC tukey HSD test were used for the statistical analysis in the present study. One-way Analysis Of Varience (ANOVA) is used to study the overall varience within the groups. However it is not possible to identify the difference between the various subgroups with the help of p-value obtained from ANOVA. Therefore a specific statistical test was used for intra-group comparision.hence the Turkey test is done in order to determine which groups differ from each other. The Tukey test Honestly Significant Difference or HSD test is a post HOC test designed to perform a pairwise comparision of the means to identify the specific sub groups in which significant differential expression occurs.

The mean and standard deviation of the bacterial reduction or cell death(%) calculated from the datas are given in the Table I

From results it can be inferred that Group IIIa showed the maximum bacterial reduction(99.5%) followed by GroupIIIb (98.89%). Group II showed the least bacterial Reduction - GroupIIa (82.81%) and Group IIb (79.2%). Group I which was the chemomechanically prepared Group showed bacterial reduction of 88.8%.

Datas were analysed using ANOVA followed by Tukey HSD test. On comparision of surviving fraction and cell death between the groups using one way ANOVA, Table I shows that ($p=0.000$) for surviving fraction and cell death which implies statistically significant difference between the Groups. ($p<0.001$). Table II also shows the mean of the surviving fraction and cell death which shows that treatment in Group III (CMP + PAD) was better than Group I (CMP alone) which was better than Group II (PDT alone).

Table I : *Surviving fraction* and *Cell death* – comparision between groups

	SURVIVING FRACTION(%)	**CELL DEATH(%)**
Groups	**Mean ± SD**	**Mean ± SD**
Group I	11.25 ± 1.48	88.80 ± 1.18
Group IIa	17.25 ± 3.61	82.81 ± 3.62
Group IIb	20.80 ± 2.76	79.20 ± 2.76
Group IIIa	0.50 ± 0.42	99.50 ± 0.16
Group IIIb	1.11 ± 0.59	98.89 ± 0.59

$p<0.001$**

** denotes significant at 1% level.

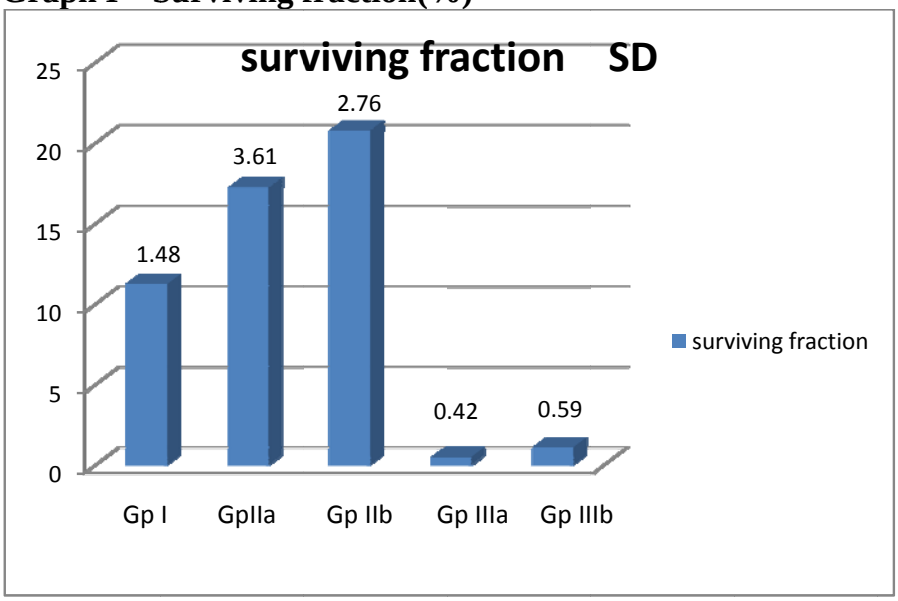
Proceeding with Tukeys HSD test , the difference is statistically significant between the Sub groups IIa and IIb(p=0.006). Sub Groups IIIa and IIIb was not statistically significant(p=0.970).Table II.

Table II – *surviving fraction* and *Cell death* -comparision within groups.

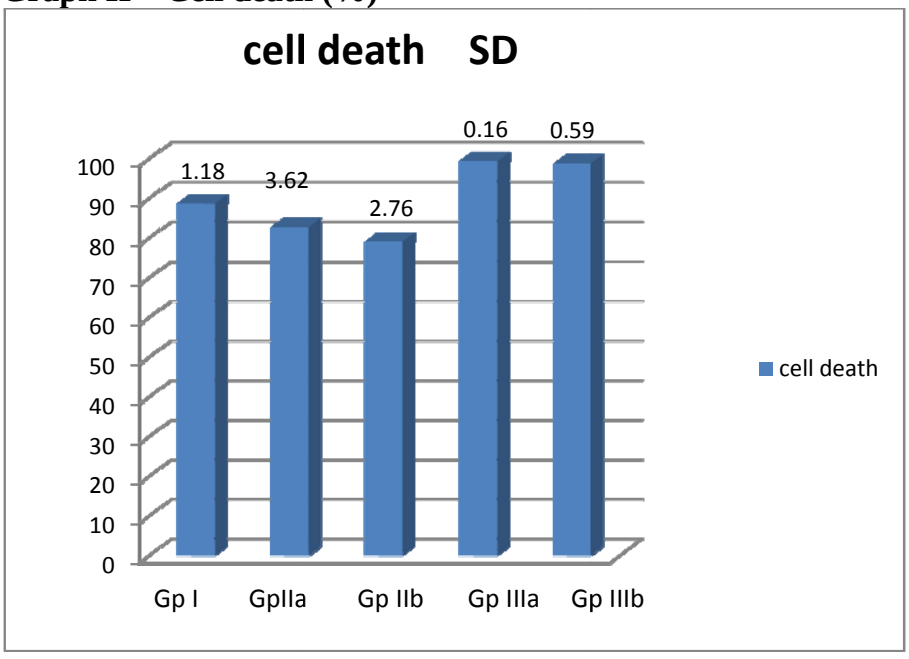
	SURVIVING FRACTION(%)		**CELL DEATH(%)**	
Groups	Mean difference	p-value	Mean difference	p-value
Group IIa & IIb	-3.5500*	0.006*	3.6100*	0.005*
Group IIIa & IIIb	-0.6100	0.970	0.5100	0.984

* denotes significant at 5% level.

Graph I – Surviving fraction(%)



Graph II – Cell death (%)



DISCUSSION

The success of endodontics is directly influenced by the elimination of microorganism in infected root canals. It is well known that microorganisms colonizing in oral environment can be conducive to pulpal and periapical pathosis. The purpose and ultimate goal of endodontics is to eliminate the bacterial infection in the root canal system and allow healing of apical periodontitis. Primary root canal therapy is a highly predictable procedure, However inability to sufficiently disinfect the root canal system may lead to failure of root canals or persistant apical pathosis.[14]

Studies have shown that flora of infected root canals comprised number of microbial species, predominantly gram positive ones. Polymicrobial infections and obligate anaerobes were also frequently found in infected canals.These organisms were predominantly gram positive cocci,which can result in endodontic infections and failure*(E Ercan et al 2006)*[14].

The most frequent genera of microorganisms isolated from root canal with necrotic pulp *were Prevotella, Fusobacterium, Lactobacillus, Streptococcus, Clostridium, Peptostreptococus* and *E-*

Faecalis. These bacteria are predominantly anaerobic and gram positive.On the other hand microbial findings of failed endodontic treatment have reported a very limited assortment of microorganisms with predominantly facultative anaerobic gram positive species, especially *E-Faecalis* and fungi such as *candida albicans*.

E. faecalis is rarely found in infected but untreated root canals. It is resistant to most of the intracanal medicaments, particularly to calcium hydroxide dressings, probably due to its ability to regulate internal pH with an efficient proton pump. *E. faecalis* can survive prolonged starvation. It can grow as a mono-infection in treated canals in the absence of synergistic support from other bacteria. In spite of the current focus of attention, it still remains to be seen, in controlled studies, that *E. faecalis* is the pathogen of significance in most cases of failing endodontic treatment[27].

The prevention or healing of endodontic pathology depends on a thorough chemo-mechanical cleaning and shaping of root canals before a dense root canal filling with a hermetic seal is done.*(Pilar Baca)*[4]. Chemo-mechanical preparation includes debriding

the infected dentinal walls of root canal system using files in a sequence and use of irrigants. Ideal irrigant or combination of irrigants kills bacteria, dissolves necrotic tissue, lubricates canal, removes smear layer and does not irritate healthy tissue. Most commonly used irrigants are hydrogen peroxide, sodium hypochlorite (2.5-5.2%), EDTA 15-17%, chlorhexidine (0.2%), MTAD, etc [49]

Complete chemomechanical preparation may be considered an essential step in root canal disinfection, however total elimination of bacteria is difficult to accomplish. To overcome this intracanal medicaments were introduced to eliminate the surviving bacteria. According to *Oguntevi* bacteria inside the dentinal tubules may constitute an important reservoir from which the root canal infection or reinfection may occur during and after endodontic treatment .Occasionally these remaining bacteria may cause persistent infection that jeopardizes the outcome of endodontic therapy. Bacteria located inside the dentinal tubules are protected from the effects of host defence cells and molecules, systemically administered antibiotics and chemomechanical preparation. Therefore treatment strategies that was directed towards the elimination of these infections are necessary and included intracanal medicaments

like calcium hydroxide .[37]

Action of Calcium hydroxide is mainly by increase in ph in the root canal system by release of hydroxyl ion . But certain bacteria such as *enterococci* tolerate very high pH value, varying from 9-11. Several studies have attested the inefficiency of calcium hydroxide in eliminating the bacterial cells inside the dentinal tubules. *Haapasalo and Orstavik (1987*) reported that calcium hydroxide paste failed to eliminate, even superficially, *E.faecalis* in tubules.[37]

Bacteria colonising necrotic tissue in ramification, isthmuses and irregularities are also protected from the action of calcium hydroxide. Therefore a short term dressing with calcium hydroxide appears to eliminate bacterial cells in direct contact with this substance such as bacteria located in the main root canal or circumpulpal dentin. These areas are also commonly affected by chemomechanical procedure. [37] Since there is a marked decrease in the prognosis of endodontic treatment, *Jacob Lee Fimple et al*[15] sought adjuncts to standard endodontic antimicrobial procedures that may increase the effectiveness of orthograde endodontic treatment or retreatment.

Contemporary techniques for root canal disinfection consist of ultrasonics and lasers used as an adjunct along with the conventional chemomechanical preparation. Ultrasonic devices were first introduced in endodontics by *Richman (1957)* . Ultrasonically activated files have the potential to prepare and debride the root canal mechanically.

Since the time ruby laser was introduced by *Maiman (1960)* , researchers have investigated laser application in dentistry . Laser is a device which transforms light of various frequency into a chromatic radiation in the visible, infrared, ultraviolet regions with all the waves in phase capable of mobilising immense heat and power when focussed at close range . *Stern and Sognnaes (1964) and Goldman et al(1964)* were first to investigate the potential use of ruby lasers in dentistry. After initial experiment with ruby lasers clinicians started using others lasers such as argon(Ar), carbon dioxide(CO_2), Nd;YAG and Er;YAG lasers. The first lasers used in endodontics was reported by *Weichman and Johnson (1971)* who attempted to seal the apical foramen in vitro by means of high power CO_2 laser. Since then attemps have been

made to seal the apical foramen using Nd:YAG laser *(Weichman et al,1972).* [48]

Lasers have been available commercially in dentistry since 1990. The various application of lasers include caries detection(Diagnodent), diagnosis of pulpal blood flow, in the treatment of dentinal hypersensitivity, pulp capping, pulpotomy, smear layer removal, root canal sterlization, tooth preparation, enamel etching, gingivectomy, bleaching, periodontal pocket disinfection , calculus removal, lethal laser photosensitisation of root canal. [23]

In *1986 Zakariasen* and colleagues for the first time demonstrated that lasers could be used in endodontics with a good bactericidal effect. In *1995 Moritz et al* achieved a partial closure of dentinal tubules using the CO_2 laser on root canal surfaces, owing to the fact that emitted long wave infrared radiation (10,600 nm)can be transmitted into the root canal exclusively by using a rigid hollow wave guide. The canal lumen must be well prepared and laser can be used only in straight root canals. Most widely used laser in endodontics is the Nd;YAG laser which emits a wavelength of

1064 nm. Owing to the wavelengths being in the near infrared range, flexible conductors can be used in curved root canals.

This laser yields a bactericidal effect on root canal surfaces and the deeper dentin layers. Various studies have shown the high bactericidal effect of the Nd;YAG laser (*Kimura 2000*)[23] The diode laser is comparable to the Nd;YAG laser in terms of effectiveness ,it emits at a wavelength of 810 nm and as comparable bactericidal capabilities .

The efficacy of Nd ;YAG laser for photothermal disinfection has been investigated and their application was found to be safe and have potential, unfortunately Nd ;YAG laser irradiation could not render all systems bacteria free.Nd;Yag laser is not an alternative but a possible supplement to existing protocols for canal disinfection as the properties of laser lights may allow a bactericidal effect beyond 1mm of dentin.[5]

All these high power laser function by dose dependent heat generation , but, in addition to killing bacteria they have the potential to cause collateral damage such as charring of dentin, ankylosis of root, melting of cementum, root resorption, and periradicular necrosis. To overcome these problems a new antibacterial

strategy that involves the combination of a non toxic photosensitiser (PS) and a laser light source within visible region ie, between 400 to 700 nm) was introduced which is termed as photoactivated disinfection (PAD). *B Dickers et al (2009)*[11] demonstrated that after 150 sec of PAD irradiation the average temperature rise was 0.16 ± 0.08ºC, the recorded values were lower than 7ºC which was within the safety level for periodontal injury. So use of PAD in root canals could be considered harmless for periodontal tissues.

PAD is a medical treatment that utilizes light to activate a photosensitizing agent(PS) in the presence of oxygen. The exposure of the PS to light results in the formation of oxygen species, such as singlet and free radicles, causing localized photo damage[45]. Photodynamic antimicrobial chemotherapy (PACT) represents an alternative antibacterial, antifungal and antiviral treatment for drug resistant micro-organisms. It is unlikely that bacteria would develop resistance to the cytotoxic action of singlet oxygen or free radicles. Application of PAD are growing rapidly in treatment of oral cancer, bacterial, fungal infections and diagnosis of malignant transformation. [24]

PAD requires a source of light that activates the PS to low power visible light at a specific wavelength. Most of the PS are activated by red light between 630 and 700 nm. In the past, PS activation was achieved via a variety of light source, such as argon pumped dye lasers, potassium titanium phosphate (KTP) or Nd;YAG pumped dye laser. All these laser systems are complex and expensive. At present diode system lasers are easy to handle, portable and cost effective and are used predominantly.[24]

The long-term use of chemical antimicrobial agents, however, can be rendered ineffective by resistance developing in the target organisms. Photo-activated disinfection (PAD) is being investigated for treatment of root canal infections. In most of the studies effect of PAD alone has been investigated and efficacy of treatment is poor as bacterial regrowth has been observed and also further studies are required against more clinically relavent organisms such as *E-Faecalis*[18].

The aim of the present study was to explore the efficacy of photo-activated disinfection in reduction in CFU's of E-Faecalis and the objective was to compare its efficacy with conventional endodontic treatment and also a combination of conventional endodontic

treatment along with photo-activated disinfection.

53 freshly extracted human maxillary incisors with straight canals, extracted for periodontal reasons, were taken for the study. The teeth were cleaned using an ultrasonic scaler and were stored in solution of 0.5% chloramine in water at 4°C until employed in the experiment. The teeth were decoronated to 14mm for standardization of length. Canals were prepared following hybrid technique to MAF size 40 with K-files. The root canals were irrigated and cleaned with 2.5% sodium hypochlorite, 17% EDTA and saline as irrigants as described by *Aguinaldo S Garcez et al (2000)*[18]. All teeth were sterilized to ensure root canals were free from contamination before inoculation with strains *of E-Faecalis.*

Then the teeth were divided into 3 main groups, Group I consisted of (n=11) samples(10 experimental+1 control), Group II(n=21) samples (1 control)and group III(n=21) samples (1 control), Group II and Group III were further divided into 2 subgroups, Group IIa ,IIb and Group IIIa, IIIb.

E-faecalis (ATCC-29212) was used as the test organism as this gram positive facultative anerobic bacterium is the most common isolate found in failed cases. *Almyroudi et al 2002*[3] found it easy to maintain and culture *E-Faecalis* under laboratory condition although this organism makes up a small percentage of the root canal flora. It may be favoured by ecological challenges and establish infections difficult to treat and demand for retreatment.

In Group I, only chemomechanical preparation was done using NaOCl 2.5% and saline till MAF 60 size, to simulate the clinical situation. 2.5% NaOCl was used as the irrigant which was a potential antimicrobial agent used in conventional root canal therapy as described *by Garcez et al (2007)*[18],which showed that chemomechanical preparation alone reduced the bacterial load by about 90%.

NaOCl is an oxidizing and hydrolysing agent. It has bacteriocidal and proteolytic actions and dissolves protiens. NaOCl have been used as irrigant as early as 1920's. Concentrations ranging from 0.5% to 5.2 % have been recommended for use in endodontics.[9] In

Group I the bacterial reduction of about 88.8% or antibacterial action was solely because of chemomechanical preparation.

In Group II all the teeth were further prepared apically till MAF 60 size to allow the fibro-optic probe of 600 µm to reach till the apex. The root canals were irrigated with 17% EDTA for 2 min followed by irrigation with saline to remove the smear layer as described by *Bergmans et al,2008*[6]. All the prepared samples were autoclaved to remove the presence of microorganisms within the canal system before inoculation with the microorganism, *E-Faecalis*.

In Group IIa and Group IIb, PAD treatment was done with a 670 nm diode laser because chlorine based PS have got the best absorption in this wave length . The 600µm fiberoptic gave a power density of 65 mw, the total energy fluence dose was 12.6 J/sec which was used for disinfecting canals for a period of 4 min and 2 min in Group IIa and Group IIb respectively. This was enough to activate the photosensitizer as described by *Fimple et al 2008*[15], in which 100 mw for 5 min was considered enough for the disinfection of the microorganisms in the canal.

In Group II the photoactivated disinfection alone reduced the bacterial load by about 82.81% for 4 min exposure and 79.20 % for 2 min exposure to laser. Laser in itself is not particularly lethal to bacteria, but aids in photoactivation of oxygen-releasing dyes which tag the bacteria.

Singlet oxygen released from dyes causes cell membrane and DNA damage to micro-organisms, which was the reason for bacterial reduction in this group[24]. In Group IIa and Group IIb there was significantly difference in the bacterial reduction because the efficiency of bacterial killing was more with more time of exposure to laser beam.

In Group III chemomechanical preparation was done initially using 2.5% NaOCl and saline alternatively which reduced the initial bacterial load by about 89%. This was in accordance to studies done by Garcez et al 2008 [19] which reduced the bacterial load by about 90% when only conventional endodontic treatment was done.

Group III was further treated with PAD for 4 minutes and 2 minutes for Group IIIa and Group IIIb respectively which reduced

the bacterial load by 99.5% and 98.89% respectively which was similar to studies done by *Silva Garcez et al 2006*[17] where he suggested that PAD used as an adjunct to the conventional endodontic treatment can lead to reduction of pathogens in a short period of time.

The initial reduction in bacterial load in Group III was due to the bacteriocidal action of the 2.5% NaOCl used in the chemomechanical preparation. The remaining viable bacteria after the chemomechanical preparation, were killed by the photoactivated disinfection.

The fiber was initially placed 2mm short of apex and moved gradually towards the middle and cervical 3rd of root canal to impart thorough disinfection of the canal. These movements were repeated approximately 6 times per as minute described by *Garecez et al 2007*.[18]

PAD involves 3 components light, photosensitizer and oxygen. Upon irradiation with light of a specific wavelength PS undergoes a transition from a low energy ground state to an excited singlet

stage. Subsequently PS may decay back to its ground stage, with emission of fluorescence, or may undergo transition to an higher triplet stage. The triplet state can react with endogenous oxygen to produce singlet oxygen and other radical species, causing a rapid selective destruction of target microorganism[24]. There are two mechanism by which the triplet state photosensitizer can react with biomolecules. Type 1 involves electron/hydrogen transfer from the PS, producing ions, or electron/hydrogen removal from a substrate molecule to form a free radical. These radicals react rapidly with oxygen, resulting in the production of highly reactive oxygen species (superoxide, hydroxyl radicals, hydrogen peroxide). Type II reaction produces the electronically exited and highly reactive state of oxygen known as singlet oxygen. In PAD it is difficult to distinguish between two mechanisms[38].

In the present study, chlorine based PS chlorine p6 was used as it is anionic in nature and was more effective against Gram positive microorganisms like *E-faecalis*. Various PS used in PAD are acridine orange, methylene blue , porphyrin derivative (HPD) (photofrin), 5–amino levunic acid (ALA), chlorine derivatives such as chlorine p6, chlorine e6[24]. The photosensitivity of bacteria

appears to be related to the charge of the sensitizer. In general neutral or anionic photosensitizer bind effectively to and inactivate gram positive bacteria while they bind to some extent to the outer membrane of gram negative bacteria. Relatively, a porous layer of peptidoglycan and lipoteichic acid outside the cytoplasmic membrane of gram positive species allows the photosensitizer to diffuse into the sensitive sites. [21]

The outer membrane of gram negative bacteria acts as a physical and functional barrier between cells and its environment. Affinity of negatively charged PS for gram negative bacteria may be enhanced by linking the PS to a cationic molecule[40,24] (eg- poly –L- lysine–chlorine p6). A PS that is taken up slowly by micro-organism may cause only cell wall damage after activation with light , whereas nucleic acid strand breakage, will be apparent on longer incubation time of PS.[47]

Results of the present study suggested that combination of the conventional endodontic procedure followed by PAD can reduce the bacterial load of E-Faecalis by about 99.5%. The chemomechanical preparation alone reduced the bacterial load by about 88.9% while

PAD alone reduced the bacterial load by about 82.81%. The results were almost similar to study by *Garcez et a l 2007*[18], in which combination of chemomechanical preparation and PAD reduced the bacterial load *(P-aeruginosa and P-mirabilis)* by about 99%.

There are two mechanisms of action that have been proposed for lethal damage caused to bacteria by PAD,(i)DNA damage (ii)damage to cytoplasmic membrane, allowing cellular contents or inactivation of membrane transport systems and enzymes. There is good evidence that treatment of bacteria with PS and light leads to DNA damage. Breaks in both single-stranded and double-stranded DNA, the disappearance of the plasmid super-coiled fraction have been detected in both Gram positive and Gram negative species after PAD. There is some evidence that PS that can more easily intercalate into double-stranded DNA can easily cause damage. Guanine residues have been shown to be more easily oxidized. [21]

The alteration of cytoplasmic membrane proteins has been shown by *Valduga et al and bertoloni et al.* Disturbance of cell wall synthesis and appearance of a multilamellar structure near the septum of dividing cells, along with loss of potassium ions from

cells was reported by *Nitzan et al*. The singlet oxygen species also react with molecules involved in maintenance and structure of cell wall, membrane such as phospholipids, peptides. Thus inactivation of membrane enzymes and receptors is also possible.[47]

The results of this present study suggests that the use of PAD as an adjuvant to the conventional endodontic treatment leads to a statistically significant further reduction of bacterial load and in particular reduces the amount of bacterial regrowth after 24 hours compared to either treatment alone. The initial bacterial load was reduced initially by the conventional endodontic procedure and the remaining viable bacteria was disinfected by PAD.

Further studies invivo especially in retreatment cases are required to validate the use of PAD as an adjunct to conventional chemomechanical preparation of the root canal. The effect of PAD and Various PS may help in complete eradication of all bacteria and ensure successful endodontic treatment.

`

SUMMARY

The purpose of this study was to compare the efficacy of Photo-activated disinfection(PAD) with standard endodontic treatment alone and combination of standard endodontic treatment and PAD as an adjunct against strains of E-Faecalis.53 extracted maxillary central incisors were used for the study. Instrumentation sequence consisted of Gates Glidden drills 4, 3 and 2 for coronal, followed by an apical preparation till MAF 40 size with K-files, using hybrid technique and the root canals were irrigated and cleaned with 2.5% sodium hypochlorite and 17% EDTA solution. Teeth were divided into 3 Groups, Group I(CMP), Group II(PAD alone), Group III(CMP + PAD).In Group I conventional chemomechanical preparation(CMP) was done using 2.5% NaOCl as irrigant. In Group II disinfection was done using diode laser of 670nm and a power output of 65mw.In Group III, conventional endodontic treatment was done initially, followed by PAD. Aliquots from the experimental groups were plated on TSB agar plates and CFU's were counted to see for surviving bacteria. It was found that the Group III had the maximum amount of bacterial killing (99.5%) compared to the other groups.

CONCLUSION

Within the limitations of the present study, it can be concluded that

(i) Photo-activated disinfection used along with chemomechanical preparation reduced the bacterial load of E-Faecalis by 99.5% at 4 minutes and 98.89% at 2min.

(ii) Chemo-mechanical preparation alone could reduce the bacterial load of E-Faecalis to 88.8% approximately.

(iii) Photo-activated disinfection could not eliminate all the bacteria in the root canal. It could eliminate only about 82.8% of E-Faecalis at 4 min and79.2% at 2 min. Hence PAD can be recommended as an adjunct following cleaning and shaping procedure to ensure thorough disinfection and sterilization of root canal system.

(iv) The diode laser at 670nm with 65mW output along with the photosensitizer chlorine p6 were effective for good disinfection of the root canal.

References

Journal references-

1.Aghahosseini,Fateme,Leila Ataie-Treatment of oral lichen planus with photodynamic therapy mediated with methylene blue ,a case report.*Oral medicine and pathlogy.2006,11,126-9.*

2. Allison Ronn R,Gordon H Downie,Rosa Cuenca- Photosensitizer in clinical PDT , *Journal of Photodiagnosis and Photodynamic Therapy, 2004,1,27-42.*

3.Alexandra Almyroudi,D Mackenzie,W P Saunders- Effectiveness of various disinfectant used as endodontic intracanal medications.*JOE,2002,vol28,3,163-167.*

4.Baca pilar,Javier C Lavero- Effect of chlorhexidine thymol varnish on root caries in a gediatric population.*JOE Jan 2007,vol33,no.1,*

5. Bergmans L,Pmoisiavdis,W Teugheles,B Ban Meerbeek,M Quirymen-Bacterial effect of Nd;YAG laser irradiation on some endodontic pathogens.*IEJ 2006,39,547-557.*

6. Bergmans L,P Moisiadis,B Huybrechts,B Vanmeerbeek,M Quirynen,P Lambrechts-Effect of PAD on endodontic pathogens.*IEJ 2008 41,227-239.*

7. Bonsor SJ,R Nichol ,TMS Reid ,GJ Pearson-photoactivated disinfection in endodontics,*British dental journal 2006,200,337-341.*

8. Bose Biplap,Alok Dube-Photodynamic efficacy of chlorine p6;a ph dependent study in aqueous and lipid environment.*Journal of photochemistry and photobiology 2008,93,32-35.*

9. Clarkson Roger M ,Alex J Moule –Sodium hypochlorite and its use as an endodontic irrigant *Australian Dental Journal 1998, 43(4).*

10. Hamlin Michael,Tatiana N Demidova-Effect of cell photosensitizer binding and cell density on microbial photoactivation.*Antimicrobial agents and chemotherapy June 2005,2329-2335.*

11.B Dickers,M Lami,P Mahler-Temperature rise during PAD of root canals .*Laser medicine science,2009, 24,81-85.*

12. Dube Alok ,Sulbha Sharma,PK Gupta –evaluation of chlorine p6 for photodynamic treatment of squamous cell carcinoma in hamster cheek pouch model.*J.Oral Oncology 2006,42,77-82.*

13. Eldeniz AU ,F Ozer,H Hadimli,O Erganis-Bacterial cidal efficacy of Er,Cr:YSGG laser irradiation against E.faecalis compared with NaOCl irrigation.*IEJ 2007 40,112-119.*

14. Ercan E ,M Dalli,Yavuz-investigation of microorganisms in infected root canals. *Bio Technol and Bio Technol,2006-20-2.*

15. Fimple Jacob Lee,Carla Raquel,Fontana,Federico Foschi,Karrin *Ruggiero*-Photodynamic treatment of endodontic polymicrobial infection *JOE 2008,34,(6),728-734.*

16.Fonseca MB,Junior PO,Pallota RC,Filho HF-PDT for root canals infected with E.Faecalis.*Photomed laser surgery,2008,june26(3),209-213.*

17.Garcez Aguinaldo Silva,Sylvia Christina,Jose Louis,Antonio Olavo Martha Simoes –Effeciency of NaOCl and laser assisted photosensitization on reduction of *E.faecalis.OOOE,2006,102,E93-98.*

18.Garcez Aguinaldo Silva,Martha S Ribeiro,George P Tegos,Michael R Hamlin-Antimicrobial PDT combined with conventional endodontic treatment to eliminate root canal biofilm to eliminate root canal bio film infection. *Lasers in surgery and medicine.2007,39,59-66.*

19. Garcez Aguinaldo Silva,Sylvia Newnez,Michael R Hamlin,Martha Simoes-Antimicrobial effect of PDT on patients with necrotic pulp and periapical lesion.*JOE Feb 2008,Vol 34,138-142.*

20. Gordon Wanda,Fernando Meza,Rony Nisan,Roy H Steven- Antimicrobial efficacy of ErCrYSGG laser with radial emitting tips on root canal dentin walls infected with *E,faecalis.JADA 2007 ,138,no.7,992-1002*

21.Hamblin Michael R ,Payyaba Hassan –photodynamic therapy –A new antimicrobial approach to infectious disease , *Photochem,Photobiol,Sci,2004,3,436-450.*

22. Kessel David and Yuluo –Photodynamic therapy : mitochondrial inducer of apoptosis , *Journal of cell death and differentiation 1999,6, 28-35.*

23. Kimura,Y P wilders,K Matsumoto lasers in endodontics-a review *IEJ 2000,33, 173-185.*

24. Konappa K and T Goslinski-Photodynamic therapy in dentistry. *Journal of dental research.2007,86(8),694-707.*

25. **Lana MA,Riveiro Sobrinho,Garzia GD ,Silva BKC,Hamdan JS, JR Carvalho** -Microorganisms isolated from root canal presenting with necrotic pulp and there drug susceptibility, *Brazillian endodontic journal, april 2001, vol 16(2),100-105.*

26. **Lee Michael T ,Philip S Bird ,Laurence J,Walsh-** Photoactivated disinfection of root canals –A new role for lasers in endodontics ,*Australlian endodontic journal,30.*

27. Love RM – *E.faecalis* –a mechanism for its role in endodontic failure, *IEJ 2001,34,399-405.*

28. **Luksiene Zivile** -A new approach to inactivation of harmful and pathogenic microorganisms by photosensitization. *Food Tecnol.Bio Technol 2005,43(4)411-418.*

29.Macdonald and Thomas J Dougherty - Basic principles of photodynamic therapy –review IANJ, *Journal of porhyrins and Phthalocyanines*
2001,5:105-129.

30. Mahmoudpour Ali,Saeed Rahimi,Mahmmod Sina,Shahriar Shahiza-Isolation and identification of e.faecalis from necrotic root canals using multiplex ECR.*Journal of oral science 2007 Vol 49,no.3,221-227.*

31. Maisch Tim,Jurgan,Baier,Barbara,Franz,Max Maier,Michael Landthaler-Role of singlet oxygen and oxygen concentration on photodynamic inactivation of bacteria. *Applied physical science and microbioly,2007,April 24,vol 104,no.17,7223-7228.*

32. Schoop Ulrich,Johannes Kimscha,Johann Wernisch-Use of ErCrYSGG laser in endodontic treatment. *JADA 2007,138,no.7,949-955.*

33. Seal GJ ,D Spratt ,M Bhatti and K Gulabiwala – An invitro comparison of bactericidal efficacy of lethal photosensitization or sodium hypochlorite irrigation on streptococcus intermedius biofilms in root canal.*IEJ 2002 ,268-274.*

34. Sharma Mrinalini,Alok Dube ,Harsha Bansal and PK gupta –effect of ph on uptake and photodynamic action of chlorine p6 on human colon and breast adenocarcinoma cell lines .*Journal of Photochem,Photobiol,Sci,2004,3,231-235.*

35. **. Sharma Mrinalini ,K Sahu ,Alok Dube ,PK Gupta-** Extracellular ph influences –mode of cell death in human colon ,adenocarcinoma cells subjected to PAD with chlorine p6,*Journal of photochemistry and photobiology,2005,81,107-113.*

36. **Sharma Mrinalani ,Livia Visai,Francesca Vraghera,PK Gupta**-Toluedene mediated photodynamic effect on staphylococci biofilms. *Journal of antimicrobial agents and chemotherapy, Jan 2008,299-305.*

37. **JF Siqueira Jr and HP Lopes** –Review –Mechanism of antimicrobial activity of calcium hydroxide *, IEJ 1999, 32,361-369.*

38. **Sibata CH ,NL Oleinick and TJ Kinsella** – photodynamic therapy;A new concept in medical treatment ,*Brazilian Journal of medical and biological research 2000,33:869-880.*

39. **Siqueira JF JR** –Review-Aetiology of root canal treatment failure : Why well treated teeth can fail *IEJ 2001, 34,1-10.*

40. Soukos Nikolas S ,Laurie Ann ,Michael R Hamblin ,Sigmund S and Tayyaba Hasan – Targeted antimicrobial photochemotherapy, *Journal of Antimicrobial agents and chemotherapy, Oct 1998,p2595-2601.*

41. Soukos Nikolaos S ,Stephen E,Sigmund S, Apostolos G Doukas-Photodestruction of human dental plaque bacteria:enhancement of photodynamic effect by photomechanical ways in an oral biofilm model. *Lasers in surgery and medicine,2003,33,161-168.*

42. **Nikolaos S Sukose ,Peter Shin Yao ,Jason T karriann,Ruggiero**-PDT for endodontic disinfection.*JOE 2006, 32,1979-984.*

43. Vandersluis LWM,Mversluis,MK Wu,PR Wesselink-Review of literature-passive ultrasonic irrigation of root canal.*IEJ 2007 40,415-426.*

44. Vivacqua N Gomes,ED Gurgel-Filho,FJ Susa-Filho- Recovery of E.faecalis after single or multiple visit root canal treatments carried out in infected teeth,*IEJ,2005,38,697-704.*

45. Walsh LJ –Current status of low level lasers therapy in dentistry, *Australian Dental Journal 1997,42 (5):302-6.*

46. Walsh LJ –Review –Current status of laser application in dentistry, *Australlian dental journal, 2003,48(3)146-155.*

47. Wainwright Mark – Review – Photodynamic antimicrobial chemotherapy (PACT) *Journal of Antimicrobial chemotherapy, 1998,42,13-28*

48. Williams Jill A ,Gavin ,J Peerson,M.John Colles – Antibacterial action of PAD used on endodontic bacteria in planktonic suspension and in artificial and human root canal. *Journal of dentistry 2006 ,34,363-371.*

Text book references.

49. Cohen - Pathways of pulp-ninth edition.